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## Editorial

### Hidden Hazards of Cardiac Pacemakers

**I**N A RECENT editorial reviewing the utilization of electric pacemakers for Stokes-Adams disease, Zoll and Linenthal<sup>1</sup> stated, "Direct electrical stimulation also carries a risk of ventricular fibrillation from technical accidents." In succeeding sentences they mentioned having knowledge of several instances in which transient fibrillation was inadvertently produced, and emphasized the danger to the patient of attaching a number of different types of electrical apparatus to him; they pointed out specifically that, when a pacemaker is used, no such apparatus should be attached to wires leading to the heart until all instruments have been properly grounded.

The dangers of cardiac pacemakers have thus been clearly pointed out, but it is believed that some further amplification and emphasis are justified with a subject title to draw attention of the profession to a hazard that might be readily overlooked. That the problem has been encountered is exemplified by the short report of fatal shock from a cardiac monitor<sup>2</sup> and by the discussion of Furman and associates<sup>3</sup> on intracardiac pacemakers in the control of heart block; in this discussion they mention that three patients died as a result of ventricular fibrillation believed induced by electric leakage from line

current. Their investigation revealed minute leaks of alternating current via the pacemaker, oscilloscope recorder, and fluoroscope.

Since the introduction of electrocardiographs with various stages of amplification and with the power derived from house current, it has been suspected that a potential hazard might be present. This was thought to be particularly true if the patient was grounded to the ground wire of the house circuit—if the plug was reversed, the patient was "charged" with the potential of such an outlet. Ten years ago, when the advisory committee on electrocardiographs of the Council on Physical Medicine and Rehabilitation of the American Medical Association was active, Dr. H. B. Williams,<sup>4</sup> who might be called "the father of American electrocardiographs," believed that the new machines carried a definite hazard but it was thought that he had a natural bias against the modern amplifying type of electrocardiographs. His emphasis on the nature of the wiring did draw many people's attention to the current that a person could obtain from the chassis of one type of electrocardiograph if another part of his body was grounded. With the introduction of intracardiac and epicardial electrodes, his fears of a Cassandra nature at that time now become stark realities.

The physician may wish to utilize direct cardiac electrodes in the study of the activation of a human heart at the time it is exposed

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during a surgical procedure, or he may utilize the potential recorded from an intracardiac or epicardiac electrode to determine its position or the nature of its contact with the myocardium. If an electrode has been placed in the right side of the heart via a venous catheter, an easy way to determine whether it is in the pulmonary artery, right ventricle or right atrium is from an electrocardiogram inscribed from it. From a surface electrode one can obtain potentials showing the amount of injury or the magnitude of the potential indicating the nature of the muscle contact. In patients who have complete or incomplete heart block, either from disease or from a surgical procedure, and who are connected to a monitor or a monitor pacemaker, it may be interesting or desirable, or both, to obtain electrocardiographic records. The cryptic dangers inherent in such a procedure must be recognized. It should be an axiom that if a combination of errors could be made by nursing, house or consultative staff, eventually an accident might take place irrespective of the intelligence and basic training of such persons. One should know the procedures that are safe and should not depart from protocol into areas where one is ignorant of the electric circuits and possible current leaks.

To document the nature of the current that could be obtained from a popular type of portable electrocardiograph,\* such apparatus was improperly connected to the power supply by turning the plug, and the current from right-leg electrode was applied to the exposed heart of an experimental animal. Ventricular fibrillation was regularly produced after the current had been flowing for 2 or 3 seconds; each time, with stoppage of the current, the heart could be resuscitated by a single shock. In one trial, touching the electrode to the atrium caused transient atrial fibrillation. The current flowing from this "right-leg electrode" was measured in the engineering laboratory and was found to amount to 0.64 milliamper; when viewed on the oscilloscope, it was practically a perfect sine wave. These

findings are in accord with those known to the research laboratory of electrocardiograph's manufacturers<sup>5</sup> who are fully cognizant of the facts reported and cooperative in their attitudes.

To hypothesize a dangerous situation in the human, one could imagine an investigator, in initiating a study of the potential from cavities or surfaces of the heart, inadvertently connecting the machine improperly to the wall plug and using the right-leg electrode as an exploring wire. Such ineptitude appears most improbable; indeed, a more likely hazard would be one wherein the intracardiac electrode is grounded and the electrocardiographic machine attached incorrectly so that the current would be flowing from the right leg to the intracardiac electrode. It is important to note that an independent ground from the chassis of the electrocardiograph would obviate the danger but the necessity of remembering to use this remains an essential problem.

The question that has undoubtedly arisen in the reader's mind is whether accidents have actually occurred in the medical institution with which the writer is associated. This proper inquiry cannot be answered categorically, but the suspicion was strong that it happened in one particular death occurring in August, 1959, at the time a pacemaker powered from the house circuit was in operation and an electrocardiogram was being taken. It is believed that since that time the regulations concerning the taking of electrocardiograms have been sufficiently emphasized that no accidents have occurred. For purposes of inquiring into such unrecognized possibilities of death, however, Taylor,<sup>6</sup> working with Kirklin, reviewed the records of 366 consecutive patients who underwent right ventriculotomy on the latter's service during the fall of 1957 and the years 1958 and 1959. In this group of patients, myocardial electrodes were left in place in 60, of whom 26 died. In a total of four cases, including the one already mentioned, the temporal relation of the death to the taking of the electrocardiogram was sufficiently close to raise the question of in-

\*Sanborn Visocardiette.

duced death, but only in the one case already mentioned was the suspicion particularly well founded.

In brief, the danger is present when the patient or multiple types of apparatus that are being utilized are not well grounded. It may be pointed out that one does not have danger in taking electrocardiograms when transistor pacemakers are in operation, although there is the slight hazard of having a direct conductor to the heart muscle which might be utilized incorrectly in some type of study of the cardiac potentials. Furman and associates<sup>3</sup> mentioned that in their work they now use battery-powered equipment to obviate the danger of the alternating-current leaks that in their earlier studies they demonstrated to occur through various types of equipment from the house power supply.

In conclusion, it may be mentioned that in the United States,<sup>7,8</sup> approximately 1,000 deaths a year are due to accidents caused by electric current. Let us, as a profession, not increase that number. In that remarkable life-restoring gift, the pacemaker, given to the profession by the scientific laboratories, one is perhaps reminded of the easy pique of the ancient Greek gods and goddesses who despite their wondrous gifts to mortals often became angered. Athene (or the equivalent Minerva) was said to have breathed life into the first mortals and to have become the patroness of colleges and of science, but she was originally the lightning goddess. Even so, the Athenean pacemaker giving life to the patient's heart may, in a fit of pique in the presence of an-

other apparatus god, undo its good by discharging a destructive bolt of lightning.

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#### Addendum

Since the above editorial was prepared, there has appeared an additional communication (Noordijk, J. A., Oey, F. T. I., and Tebra, W. Lancet 1: 975, 1961) pointing out the hazard of intracardiac electrodes. Ventricular fibrillation occurred in two patients when an electrocardiograph was used. Resuscitation was possible in both.

The editorial does not touch on the theoretical and potentially practical problems with stimuli to the heart which is intermittently not in AV block and when a type of interference dissociation is produced. To date, this has not created any problem to my knowledge.

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The theories which embody our scientific ideas as a whole are, of course, indispensable as representations of science. They should also serve as a basis for new ideas. But as these theories and ideas are by no means immutable truth, one must always be ready to abandon them, to alter them or to exchange them as soon as they cease to represent the truth. In a word, we must alter theory to adapt it to nature, but not nature to adapt it to theory.—CLAUDE BERNARD. *An Introduction to the Study of Experimental Medicine*. New York, The MacMillan Company, 1927, p. 39.

## Sodium Dextro-Thyroxine in Coronary Disease and Hypercholesterolemia

By RICHARD J. JONES, M.D., AND LOUIS COHEN, M.D.

A STRIKING REDUCTION in serum lipids as a consequence of the administration of thyroid substance has long been appreciated. However, thyroid analogues have not been regarded as practical agents for lowering the serum cholesterol of euthyroid patients because of their more primary calorogenic and cardiac stimulating effects<sup>1, 2</sup> and because of the reported escape from this effect.<sup>2, 3</sup> More recently fairly substantial evidence has suggested that various other measures of thyroid activity, including serum lipid reduction, do not necessarily parallel the calorogenic activity of many analogues of thyroxine and triiodothyronine,<sup>4, 5</sup> and the escape of serum lipids after prolonged administration of such compounds has been found to occur only at low dosage.<sup>6</sup>

Of these analogues particular interest has centered about the dextro-isomer of thyroxine. Pitt-Rivers and Lerman<sup>7</sup> found that the calorogenic effect of the dextro-isomer was one tenth that of the naturally occurring l-thyroxine. According to Starr<sup>8, 9</sup> a dose of dextro-thyroxine 50 times the usual therapeutic dose of levo-thyroxine had a suppressive effect upon the serum cholesterol with little, if any, calorogenic effect in diverse patients showing serum cholesterol elevation. He<sup>8-10</sup> has further suggested that it has a minimal effect on the heart in comparison with a calorigenically equivalent dose of levo-thyroxine. Oliver and Boyd<sup>11</sup> have claimed that, among many com-

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pounds studied, dextro-thyroxine showed the greatest disparity between its calorogenic and hypcholesteremic effect. Larson and co-workers<sup>12</sup> found no deiodination of dextro-thyroxine in an in vitro system. However, comparative isotope studies on the disposition of d- and l-thyroxine in the rat by Tapley and co-workers<sup>13</sup> revealed not only that d-thyroxine was excreted more rapidly, but also that it was concentrated twice as much in the liver while only one sixth as much in other tissues (e.g., heart muscle) as was l-thyroxine.

### Material and Methods

Outpatients of the University of Chicago Clinics with coronary disease or hypercholesterolemia, and on whom monthly values of serum cholesterol were available for 6 months before treatment, were given 4 to 8 mg. of sodium d-thyroxine\* daily. The larger dose was used initially, but the 4 or 6 mg. dose was later used, even as the starting dose, when adverse effects on the larger dose became apparent. Basal metabolic rate, pulse rate, and blood pressure were measured initially and at least once more, after about 3 months of treatment. In addition the patients were seen in regular clinic visits and examined for signs of hyperthyroidism or worsening cardiac symptoms at appropriate intervals.

Of 20 patients initially considered for this study, one did not cooperate; three developed a sharp increase in the frequency of angina pectoris and discontinued the treatment; and one patient died suddenly at work, while in the tenth week of treatment. Two of those developing angina and the uncooperative patient were excluded; hence the study included one hypothyroid and 16 euthyroid patients, all followed for at least 10 weeks.

Total lipids and phospholipids of sera, drawn from patients in the fasting state, were measured initially, and at 3 months; the serum cholesterol was determined biweekly throughout the first 3 months of treatment. The total lipids were determined gravimetrically from a Bloor extract,<sup>14</sup> the cholesterol by the technic of Abell and co-work-

\*Generously supplied by Baxter Laboratories as Choloxin.

ers,<sup>15</sup> and the phospholipid by the Gomori technique.<sup>16</sup> In four subjects serial observations were also made on the cholesterol partition among the three lipoprotein fractions separated by two preparative centrifugations at densities of 1.063 and 1.019, as described by Havel et al.<sup>17</sup> with minor modifications described elsewhere.<sup>18</sup>

### Results

In figure 1 may be seen the time course of the serum cholesterol in all the euthyroid patients who continued the treatment for 9 weeks or longer. Except for two patients who fluctuated rather widely at first, the individual cholesterol curves showed a sharp reduction in serum cholesterol levels within 2 weeks, which was sustained for the first 3 to 6 months at an average level of about 73 per cent of the control level.

Except for one patient, each showed a statistically significant ( $p < .01$ ) reduction in mean serum cholesterol level averaging 27 per cent below his mean control level. From table 1, it may also be seen that there was a somewhat less striking reduction in serum phospholipid and total lipid values so that the cholesterol:phospholipid ratio was reduced. In table 2, the serial observations in four of our subjects suggest that the only lipoprotein fraction consistently contributing to the serum cholesterol reduction was that of density 1.019 to 1.063. The heavy density ( $> 1.063$ ) lipoprotein cholesterol was but little affected and the low density ( $< 1.019$ ) lipoprotein cholesterol was not significantly affected by this administration of sodium dextro-thyroxine except in the hypothyroid patient A.P.

### Calorigenic and Other Side Effects

All patients lost weight during the first 3 months of treatment, the average weight loss being  $3\frac{1}{2}$  lb. This loss is reflected in the increased daily basal caloric requirement, as estimated from the basal oxygen consumption, which averaged 174 calories on the 8-mg. dose and 87 calories on the 4- to 6-mg. dose. This loss was not disadvantageous to many of these patients who had been trying to lose weight for years, and some patients volunteered an increase in energy, in stamina, and in a sense

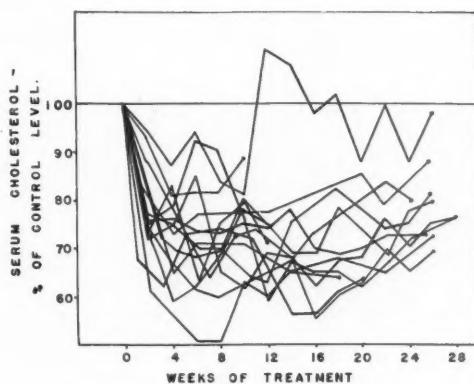


Figure 1

The per cent of the control serum cholesterol levels, plotted against weeks of treatment up to 6 months in the 15 patients listed in table 1 who took 4 to 8 mg. daily of sodium d-thyroxine for 9 weeks or longer. The control level (100 per cent) was based on the mean value for 6 preceding monthly determinations.

of well-being. Two patients developed an obvious lid lag and tremor of the tongue on the larger dose.

Much more disturbing was the increase in intensity of cardiac symptoms. One man (R.B.) developed severe classical angina of effort for the first time since a myocardial infarction 3 years earlier. Of the patients excluded from the study, one who had a stable anginal syndrome experienced a sharp increase in frequency of pain from once to several times a day, often in the absence of precipitating exertion, on the 8-mg. dose. Another whose anginal pattern had been stable for years also had a decrease in exercise tolerance and the new development of nocturnal angina. Two of these patients could tolerate the drug no better when it was reinstated 2 weeks later at 4 mg. per day. The last patient was able to tolerate a dose of 2 mg. per day of sodium-d-thyroxine, even though he still felt a questionable and subjective reduction in frequency of angina on final discontinuation of that dose. His mean serum level of cholesterol (not included in table 1) was reduced from 296 mg. per cent during the control period to 260 mg. per cent by this dose. Of the other 11 patients (including

**Table 1**  
*Response of the Basal Metabolic Rate, Weight, Pulse Rate, and Serum Lipid Levels to Administration of Sodium D-Thyroxine in 16 Euthyroid Patients*

Patient	Age	Sex	Diagnoses*	D-thyroxine dose, mg./day	Treatment interval, weeks	Before sodium d-thyroxine			After 3 months of sodium d-thyroxine								
						B.M.R. %	Body wt., kg.	Final pulse min.	Serum lipid mg. %	Cholesterin mg. %	Total lipid mg. %	Serum lipid mg. %					
M.I.B.	63	F	AS	8	14	-4	50.5	59	330	380	1495	+17	48.0	67	239	307	1095
P.E.	55	M	AS	8	17	-6	7.99	77	245	235	880	+4	76.4	79	192	222	750
V.F.	58	F	AS, PMI, CHF	8	19	-3	53.5	70	322	285	955	+11	50.6	75	234	...	...
R.G.	50	M	AS, PMI	8	14	-1	56.1	58	289	248	885	+6	53.5	61	176	205	665
M.I.	50	F	AS, PMI	8	13	-17	73.2	57	303	255	930	+9	72.3	71	219	237	700
M.B.	65	F	AS, PMI, EH, CHF	8	9	+4	49.1	59	352	305	970	+22	47.2	64	245	255	805
R.S.	52	M	PMI	8	10	-12	69.6	68	336	317	1160	-7	69.0	74	287	265	925
R.B.	59	M	PMI	8	3	-10	79.8	66	330	325	1215	-2	79.0	71	239	257	875
Average				12	-6.1	65.5	64	308	294	1061	+6.2	63.3	70	227	250	831	
H.H.	44	M	AS, PMI	6	13	-16	76.8	71	340	327	1365	-9	75.6	70	230	227	760
H.I.	44	M	NCVD	6	17	-9	64.8	63	272	255	805	+5	62.2	72	221	232	760
S.S.	47	F	AS, XT	6	26	-6	51.7	84	488	367	1345	+2	50.3	89	335	282	1010
L.L.	59	F	NCVD	6	26	-13	52.4	63	383	285	1140	-9	51.0	71	293	280	960
M. W.	60	M	AS, PMI	4	24	-4	56.9	62	346	345	1190	+4	55.4	61	276	282	920
H.C.	45	M	PMI, AD	4	13	-16	62.3	61	363	362	1455	-9	61.4	76	265	327	1345
D.G.	52	F	PMI	4	26	-11	36.8	64	336	287	1095	-7	36.7	69	244	230	875
F.B.	56	M	AS, PMI	4	18	-14	82.9	64	270	267	910	-9	82.6	59	255	262	975
Average				20	-11.1	59.3	66	341	301	1115	-4	58.2	71	265	265	951	

\* Diagnostic initials: AS, anginal syndrome; CHF, congestive heart failure; PMI, previous myocardial infarction; EH, essential hypertension; NCVD, no cardiovascular disease; XT, xanthoma tendinum; AD, Addison's disease.

† Mean of 6 or more determinations.

A.P.) afflicted with the anginal syndrome, some more severely than the above three, none had any such gross change in his pain pattern and two thought that perhaps it had improved. Actually, no intensification of the anginal syndrome occurred in those patients started on the 4 or 6 mg. daily dose, and a graded increase to full dosage was not regularly prescribed.

A return of the symptoms of congestive heart failure was noted in two women (M.B. and V.F.) after 10 weeks on the 8-mg. dose, and after 1 year on the 6-mg. dose, respectively. These symptoms were not easily controlled until the d-thyroxine was stopped and, in M.B., later adjusted to the 4-mg. dose. One patient, receiving long-term Dicumarol therapy for a previous myocardial infarction, died suddenly at work while in his tenth week on the 8-mg. dose. No autopsy was obtained, and clinical information was too limited to permit any conclusion regarding a possible relationship between dextro-thyroxine administration and his death.

In 11 patients pursuing long-term Dicumarol therapy, great variability in prothrombin times occurred during their first month of treatment. In all but three, a reduction in dosage of Dicumarol was necessary because of a prolonged prothrombin time; two of these three had a shortened time followed later by a prolonged prothrombin time, also requiring adjustment of dosage. Thus only one patient, F.B., whose response to the d-thyroxine was weak in every other way, showed no unusual fluctuation in Dicumarol requirement. None of these patients suffered a hemorrhagic episode. No equivalent disturbance in prothrombin time regulation occurred on withdrawal of the thyroxine isomer.

#### Escape of the Serum Cholesterol

Seven of these patients have continued to take the thyroxine isomer for long periods of 11 to 20 months, and their serum cholesterol is plotted as the percentage of the control level against time in figure 2. It may be seen that there is generally a return from maximum cholesterol depression in the first month to a leveling-off at a stable serum concentration for the next 6 months. In two patients

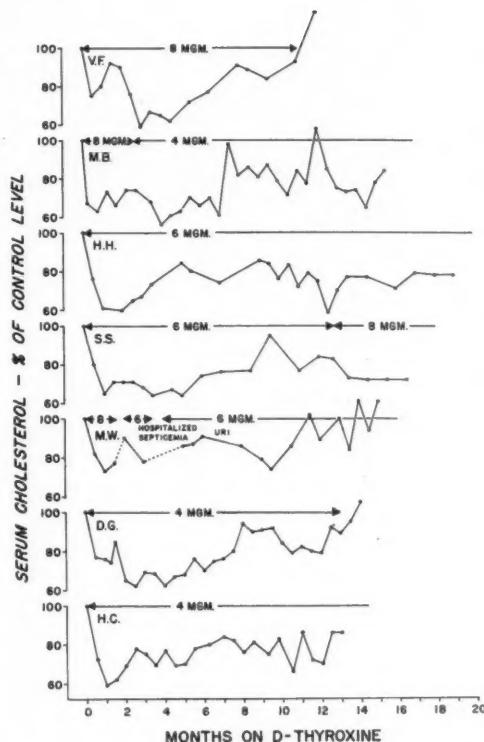


Figure 2

*Serum cholesterol variations in seven patients treated with sodium d-thyroxine for 10 to 18 months. The horizontal line above each curve indicates the mean control level for each subject, the daily dose of sodium d-thyroxine is indicated by the figures enclosed by the arrow heads, which indicate the beginning and end of each dosage level.*

(M.B. and M.W.) dosage adjustments in the first 4 months complicate interpretation, but there is a definite tendency for the serum cholesterol to be higher in the second 6 months than in the first 6 months. In all but two subjects (H.H. and H.C.) a gradually rising trend was apparent by the end of 1 year. The serum cholesterol still remained about 15 to 20 per cent below control levels, however, and an increase in the daily dose from 6 mg. to 8 mg. in S.S. restored the lower level of 12 months earlier. Always, when the drug had been terminated, a sharp rise in serum cholesterol has been noted (e.g., V.F. and D.G. of fig. 2).

Comparison between the response to so-

Table 2

*Distribution of Cholesterol among the Three Major Lipoprotein Fractions in Four Patients in Mg. per 100 Ml. Serum before and during the Administration of Sodium-d-d Thyroxine, 8 Mg. per Day*

Period Lipoprotein density	Control			D-thyroxine		
	<1.019	1.019-1.063	>1.063	<1.019	1.019-1.063	>1.063
	mg. %	mg. %	mg. %	mg. %	mg. %	mg. %
S. S.	27	378	57	42	248	43
M. B.	44	195	83	28	116	73
M. L.	34	179	64	37	109	60
A. P.*	55	301	115	15	149	59

\* $I^{131}$  induced myxedema.

dium d-thyroxine and sodium l-thyroxine was made in one patient, A.P., who had been made hypothyroid by radioiodine treatment of a hyperthyroid state. This patient was clinically myxedematous, with puffy facial edema, slow mentation, thickened speech, weight gain, and constipation on February 21, 1959, and later on December 12, 1959, when he was receiving no medication. On the latter occasion, d-thyroxine was reinstated without waiting for the basal metabolic rate to fall lower. The serial basal metabolic rate and serum cholesterol analyses may be seen in table 3. From this comparison, it may be concluded that the dose of sodium d-thyroxine required to maintain this patient in the eucaloric state is roughly 50 times that of l-thyroxine. In this particular case, the hypocholesteremic effect and calorogenic effect seem to be disproportionate: the serum cholesterol was much lower on the d-thyroxine than on the calorically comparable dose of l-thyroxine and a dose only 20 times as great still effected a lower level of serum cholesterol than was seen with the largest dose of l-thyroxine.

#### Discussion

The marked hypocholesteremic effect of thyroid-active compounds is well observed in these 16 euthyroid subjects. Our hypothyroid patient experienced a hypocholesteremic effect much more dramatic with d-thyroxine than on a calorically equivalent maintenance dose of l-thyroxine. These two observations confirm those first made by Starr.<sup>8, 9</sup> Whether serum cholesterol reduction by the d-thyroxine preparation is similarly disproportionate to its caloric effect, in comparison with

l-thyroxine, in the euthyroid patient cannot be determined from these data. However, Strisower et al.<sup>10</sup> treated 16 euthyroid subjects with roughly 1 mg. per day of an l-thyroxine preparation: about 10 times the usual replacement dose of l-thyroxine employed here. Precise caloric data were not reported but, from the observed weight loss and increase in pulse rate, their subjects, though enjoying a serum cholesterol reduction comparable to that reported here, sustained a much greater calorogenic effect.

The practical advisability of using this dextro-isomer of thyroxine for the control of serum cholesterol in euthyroid patients is somewhat impaired by the fact that it does have a weak but definite calorogenic effect. While it seems to be true that this is weaker than its hypocholesteremic effect, when compared with other thyroxine analogues, it is still capable of provoking cardiac symptoms in patients who may otherwise be quite comfortable. Even this risk might be worth taking, if the assurance were great that this hypocholesteremic effect would halt the process of atherogenesis or prevent further occlusive episodes. It is still uncertain, however, what benefit serum cholesterol reduction may provide to the patient with coronary artery disease: certainly our fatality had an unsuccessful outcome.

Our limited data on lipoprotein cholesterol concentrations suggest that, as has been demonstrated in the case of thyroid extract and l-thyroxine,<sup>3, 6, 10</sup> the suppressive effect is virtually limited to the medium density (1.019 to 1.063) beta lipoprotein. While some evidence relates hyper-beta-lipoproteinemia to

Table 3

*Basal Metabolic Rate and Serum Cholesterol on Comparable Doses of Sodium-d-Thyroxine and -l-Thyroxine in a Subject with Hypothyroidism*

Medication	Dose mg./day	Date*	B.M.R. %	Serum total cholesterol mg. %
None		10/4/58	+31	185
5 mc. I <sup>131</sup>		10/15/58	..	..
None		12/23/58	- 5	395
None		2/21/59	-23	433
Na-d-thyroxine	10	3/21/59	- 1	161
		4/4/59	0	168
		4/25/59		203
Na-l-thyroxine	0.05	5/14/59		300
		5/26/59	-12	307
		6/10/59		345
Na-l-thyroxine	0.10	8/4/59	-15	282
Na-l-thyroxine	0.20	8/15/59		227
		8/29/59	- 9	233
		10/3/59		245
		10/17/59	+ 2	235
None		11/7/59	- 9	399
		12/12/59	-15	463
Na-d-thyroxine	6	1/23/60		225
		2/27/60		198
		3/21/60		177
		4/23/60	- 4	195
		5/21/60		219
Na-d-thyroxine	4	6/25/60		202
		7/30/60		209
		9/10/60		209
		10/29/60	-10	212

\*The new dosage always started on the date above the preceding horizontal line.

atherogenesis, there are those who feel that the lighter density alpha<sub>2</sub> material ( $S_f$  20-400 or  $d < 1.019$ ) may be more important in this regard.<sup>20, 21</sup> Thus, in the present state of our knowledge, the accomplishment of such a hypocholesteremic effect with the attendant hazards recorded here does not seem warranted except perhaps in subjects with minimal or no symptoms of heart disease.

The disturbance in Dicumarol therapy as indicated by the rise and fall of prothrombin times was unexpected. No effort at initial graded dosage was made in this study, and this may have emphasized that effect. Two incomplete reports have suggested that the feeding of thyroid substance to rats did enhance the prolongation of the prothrombin times induced by a salicylate<sup>22</sup> or warfarin.<sup>23</sup> In any case, the observation suggests that patients receiving these two drugs should have frequent determinations of the prothrombin time initially.

#### Conclusion

A marked hypocholesteremic effect (27 per cent) was seen with the administration of sodium d-thyroxine given in doses of 4 to 8 mg. per day. It was primarily, as with l-thyroxine, the beta lipoprotein ( $d$  1.019 to 1.063) cholesterol that was affected. The reduction in serum cholesterol was better sustained at the higher dosage levels than at 4 mg. per day but a slight tendency for the cholesterol to rise after 1 year could be overcome by a fractional increase in dosage.

Sodium d-thyroxine showed the same capacity to elevate basal caloric requirement, enhance the frequency of the anginal syndrome, and occasionally aggravate symptoms of congestive heart failure that has been reported with other active thyroid compounds. In this study these adverse effects were seen predominantly at the 8-mg. daily dose, but even smaller doses were not always tolerated in susceptible patients and the tendency of

the serum cholesterol to return to pretreatment values was more often seen after 6 to 9 months at lower dosage levels.

A puzzling alteration in the prothrombin time of patients maintained on Dicumarol therapy was frequently observed early in the course of d-thyroxine therapy.

Although it is clear that a hyper-beta-lipoproteinemia can be partially corrected by the administration of sodium d-thyroxine, the usual caution observed in the administration of thyroid compounds to patients with heart disease is still deserved. In view of the present uncertainty of the goal of serum lipoprotein manipulations in coronary artery disease, the use of this preparation for that disease cannot be recommended without qualifications and should best be reserved for patients free of cardiac symptoms.

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# Congenital Communication of a Coronary Artery with a Cardiac Chamber or the Pulmonary Trunk ("Coronary Artery Fistula")

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THE COMMUNICATION of a coronary artery with a cardiac chamber or the pulmonary trunk, although an uncommon anomaly, is important for several reasons. It enters into the differential diagnosis of those conditions associated with a continuous precordial murmur, among them patent ductus arteriosus, aortieopulmonary septal defect, ventricular septal defect with aortic insufficiency, supravalvular pulmonary arterial stenosis, ruptured aneurysm of an aortic sinus, and congenital absence of the pulmonary valve. Moreover, the anomaly may become complicated by bacterial infection or congestive cardiac failure or both. Ordinarily, it is amenable to cure by interruption of the fistulous tract.

In this report we describe six cases observed at the University of Minnesota Hospitals and compare them with those reported in the literature.

The six patients, three male and three female, ranged in age from 17 months to 53 years (table 1).

Of the six patients, five underwent surgical treatment; all five are living. The sixth patient (case IV) did not receive surgical therapy; the diagnosis was confirmed at necropsy.

## Pathologic Features

There are two types of gross communication between a coronary artery and a

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cardiac chamber. The first type is characterized by an intact ventricular septum (or a very small ventricular septal defect) and atresia either of the pulmonary or the aortic valve.<sup>1-3</sup> Under these circumstances, and in the presence of a competent corresponding atrioventricular valve, enlarged sinusoids of the ventricle proximal to the atretic semilunar valve penetrate the myocardium. These sinusoids converge in or near the epicardium to form a single vessel leading from the obstructed ventricle. In the epicardium, this anomalous vessel joins with the usual branches of the coronary arteries. Then, blood from the obstructed ventricle is forced into the coronary arteries, which receive this blood in addition to their supply from the aorta. When pulmonary atresia is present, venous blood is therefore delivered to the coronary arteries, while when aortic atresia is present, blood fully saturated with oxygen is delivered from the left ventricle directly into the coronary arteries by way of the channels mentioned.

In all the six cases here reported the anomalous coronary arterial communication was of the second type. In it a coronary artery communicates with a cardiac chamber or pulmonary arterial trunk without other cardiac malformation, except coincidentally. In our six cases the abnormal coronary artery was the right in two cases and the left in four cases. The sites of termination of the coronary artery were as follows: (1) when the right artery was involved—right ventricle, one case (case I) and right atrium, one case (case VI); (2) when the left artery was involved—right atrium, one case (case IV), right ventricular cavity, one case (case II),

**Table 1**  
*Pertinent Findings in Six Cases of Congenital Communication of a Coronary Artery with a Cardiac Chamber or Pulmonary Trunk*

Case no.	Age (yr.)	Sex	Respiratory infections	Dyspnea	Congestive heart failure	Systolic thrill	Continuous murmur	Second sound pulmonary area	Systemic blood pressure	Electrocardiogram		Coronary artery	Site of termination
										QRS axis	Hypertrophy		
I	10	F	+++	++*	—†	+	4th LICS	Normal	100/60	-170	R.V. Diast. Overload	Right	RV
II	1 yr. 5 mo.	M	++	—	—	—	Gr. III at apex	++	140/80	+ 90°	None	Left	RV
III	53	F	+	+	++	—	Gr. III 2-3rd area	Normal	160/100	+ 60°	Left Vent.	Left	PT
IV	43	M	—	—	++	—	4th LICS	+	160/75	+ 30°	Left Vent.	Left	RA
V	19	M	—	—	—	—	3rd LICS	+	125/85	+ 90°	—	Left	PT
VI	19	F	—	+	—	+	2 RICS	+	130/60	+ 90°	—	Right	RA

RA, right atrium; RV, right ventricle; PT, pulmonary arterial trunk; LICS, left intercostal space; RICS, right intercostal space.  
 \*+, Condition present. †—, Condition not present.

and pulmonary trunk, two cases (cases III and IV).

Existing accounts report various sites of anomalous termination of coronary arteries (table 2). Although an attempt was made to review all reported cases, some may have been overlooked. In a total of 50 cases the right coronary artery was involved slightly more commonly than the left. The sites of termination were most often the right atrium or a cardiac vein, the right ventricle, and the pulmonary trunk.

A single opening is often described at the site of an anomalous connection of a coronary artery with a cardiac chamber or pulmonary trunk, but sometimes multiple connections are noted. In case V, for example, we observed three openings into the pulmonary trunk.

Characteristically, the coronary artery that enters into anomalous communication with a cardiac chamber or the pulmonary trunk is obviously enlarged, being not only dilated but elongated and tortuous. The enlarged artery also commonly shows focal saccular aneurysms with calcified walls. In one reported case<sup>26</sup> of an abnormal connection between a branch of the left coronary artery and the right atrium, the vessel did not show dilatation. Biorek and Crafoord's<sup>36</sup> case showed only a minimal dilatation, as did case V in our series..

In one of our patients (case I), a segment of coronary artery was removed at the time of surgical interruption of the fistula to the right ventricle. Histologic examination of this segment revealed marked thickening of the media with prominent muscle bundles interspersed with numerous elastic fibers. Nonspecific focal fibrous thickening was also present (fig. 1). These features are common in arteries in any site that lies proximal to an arteriovenous communication.

#### Embryology

In early prenatal life, the coronary arteries communicate with the veins through a classic capillary network; they also give off branches to the intertrabecular spaces that communi-

cate with the ventricular cavities. In later prenatal life, the intertrabecular spaces shrink to form the sinusoids, which represent communications between the veins and coronary arteries, on the one hand, and the cardiac chambers on the other.<sup>43</sup> Abnormally large connections of the type here considered appear to represent persistence of the large intertrabecular spaces, which connect with a ventricular cavity, on the one hand, and with a coronary artery, on the other.

A different explanation for anomalous communication between a coronary artery and the pulmonary trunk exists. This anomaly apparently occurs when one or several coronary arteries arise from the pulmonary trunk. The accessory artery or arteries in turn make communication with branches of one or both normally arising coronary arteries. As flow from the high pressure (aortic) area is carried into the low pressure (pulmonary) area, the communication enlarges.

#### Clinical Features

The natural history of this malformation is not well known. In most cases reported in the literature the patients reached adult life. Only occasionally did death occur in infancy or childhood.

Symptoms seem to depend upon the magnitude of the shunt. Among our patients, three gave histories of recurrent respiratory infections. Congestive cardiac failure was observed in two patients (cases III and IV) and was the cause of death in one of them (case IV). In another patient (case III), signs of congestive cardiac failure disappeared after surgical interruption of the anomalous channel.

Some patients may manifest symptoms and signs of coronary insufficiency. In case IV, the patient was a 43-year-old man whose chief complaint was angina. Obviously, in this age group it is difficult to determine whether angina is related to the malformation itself or to concomitant coronary atherosclerosis. In other cases, bacterial infection may develop in the abnormal communication.<sup>17</sup> Steinberg and associates<sup>44</sup> reviewed 22

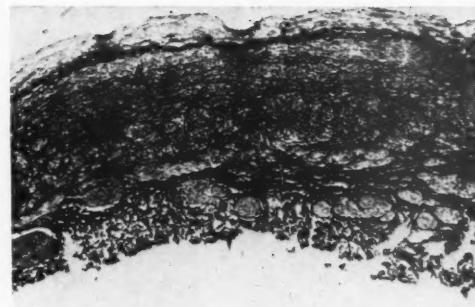


Figure 1

*Case I. Photomicrograph of right coronary artery proximal to its entrance into the right ventricle. Hypertrophy of the media. Numerous elastic fibers course between bundles of muscle. The intima (above) shows mild nonspecific fibrous thickening. Elastic-tissue stain.*

reported cases of coronary arteriovenous fistula and added one case diagnosed by angiography. In only two cases was death directly attributed to the fistula; in the others it was due to nonrelated causes.

In those cases in which the anomalous termination is into the left ventricle, only a diastolic murmur is anticipated. In cases involving the other possible sites of termination, a continuous murmur frequently occurs; in fact, it is the most consistent abnormal physical sign among reported cases, and was noted in all six cases reported here.

The location of maximal intensity of the continuous murmur seems to depend upon the site of the abnormal communication, i.e., its position usually corresponds with the anatomic position of the chamber or vessel participating in the anomalous connection. We observed the association between a continuous murmur and a systolic thrill only in two patients (cases I and VI), but this association is commonly reported in the literature. The second sound at the pulmonic area was accentuated in all six of our patients and was particularly prominent in four (cases II, IV, V, and VI). The absence of a murmur in Scott's case<sup>24</sup> is perhaps explained by the observation at necropsy that a thrombus had occluded the fistula.

**Table 2**  
*Coronary Artery of Origin and Site of Anomalous Termination; 50 Cases Reported in this Study and in the Literature*

Coronary involved	Site of termination	Cases		Source (by bibliographic number)*
		Number	Per cent of 50	
Right	Right atrium or cardiac vein	14	28	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, VI
Right	Right ventricle	8	16	16 (4 cases), 17, 13, 18, I
Right	Pulmonary trunk	6	12	19, 20, 21, 22, 23 (case 2) 24
Total right	All	28	56	
Left	Right atrium or cardiac vein	4	8	25, 26, 27 IV
Left	Right ventricle	8	16	28, 29, 30, 31, 32, 33, 34 II
Left	Pulmonary trunk	5	10	24, 35, 36, III,† V.
Left	Left atrium	3	6	37, 38, 39
Left	Left ventricle	2	4	40, 41
Total left	All	22	44	
Total left and right		50	100%	

\*Roman numerals indicate case number of cases in this communication.

†This case was reported previously<sup>42</sup>

Gasul and associates<sup>16</sup> documented with phonocardiography their observation that the diastolic component of the continuous murmur was louder than the systolic component in patients with fistulas that communicated with the right ventricle. This diastolic accentuation contrasts with the systolic accentuation usually observed in patent ductus arteriosus.

#### Electrocardiographic Features

In anomalous communication between a coronary artery and a cardiac chamber or the pulmonary trunk, the electrocardiogram may show various abnormalities, but no specific diagnostic patterns are present.

Electrocardiograms were obtained for each of our six patients. In two cases (cases III and IV), a pattern of left ventricular overload was observed. One case (case I) showed right ventricular diastolic overload, and one case (case VI) showed flattening of the T waves in all leads. In the remaining two cases no electrocardiographic abnormalities were noted.

The electrocardiographic findings seem to depend upon the anatomic situation and associated alterations in hemodynamics. If the left-to-right shunt is small and insignificant, no specific abnormal findings will appear in

the electrocardiogram. If, however, a wide anomalous communication and a large shunt exist, signs of left ventricular hypertrophy may be found. In case IV of our group, the left-to-right shunt was shown to be more than 8 liters per minute per square meter of body surface and the electrocardiogram revealed signs of left ventricular overwork. In cases in which the coronary artery is anomalously connected with the right atrium, both left ventricular hypertrophy and right ventricular volume overload pattern may occur, as in the case reported by Edwards and associates.<sup>3</sup>

Quite apart from shunts potentially affecting the electrocardiogram, the possibility of alterations resulting from associated myocardial ischemia should be considered. Usually no such alterations are present, however. In case IV of our series, although the patient presented a history of anginal pain, electrocardiogram did not reveal changes in the ST-T segments.

In one case reported by Colbeck and Shaw,<sup>9</sup> involving anomalous communication between the right coronary artery and right atrium in an 80-year-old man, the electrocardiogram showed atrial fibrillation and left bundle-branch block. At necropsy the heart weighed 554 Gm., and left ventricular hypertrophy

**Table 3**  
*Synopsis of Cardiac Catheterization Data in Four Cases in This Study*

Case no.	Right atrium		Right ventricle		Pulmonary artery		Peripheral artery	Left-to-right shunt
	Pressure (mm. Hg)	Oxygen (vol. per cent)	Pressure (mm. Hg)	Oxygen (vol. per cent)	Pressure (mm. Hg)	Oxygen (vol. per cent)	Oxygen saturation (vol. per cent)	(L./min. /M. <sup>2</sup> )
I	4/2	10.36	50/0	12.39*	20/5	12.13	16.57	1.3
II	7/2 (4)	7.69	50/0	12.36*	50/27	11.48	14.58	1.3
III	8/2	12.60	20/0	12.08	16/4	13.76*	17.7	0.6
IV	10/4 (8)	19.66*	19/2	18.98	25/12	19.87	21.74	8.7

\*Indicates anatomically identified site of anomalous termination of a coronary artery  
 (SVC = Superior Vena Cava)

and dilatation were noted. The important functional disturbance of this condition is the run-off of blood from the coronary arterial system similar to that in other arteries that lie proximal to arteriovenous or arteriovenous-like fistulas. Consequently, when a coronary artery communicates with a right-sided cardiac chamber, cardiac catheterization may reveal high oxygen saturation of the blood in that chamber receiving blood from the coronary fistula. When the abnormal vessel communicates with the left side of the heart, no abnormality in oxygen saturation occurs.

Hemodynamic studies were performed in four of our six cases. A summary of the observations is shown in table 3. In each case an increase in oxygen saturation was detected in the chamber or vessel receiving the abnormal communication (right atrium, one case; right ventricle, two cases; pulmonary trunk, one case). Calculated shunts varied from a minimum of 0.6 to 8.7 liters per minute per square meter of body surface. A similarly high value for a left-to-right shunt was obtained in one of Gasul's cases.<sup>16</sup> Pulmonary arterial and right-sided intracardiac pressures were within normal limits in two cases.

In the anomaly under discussion, diversion of aortic blood into the coronary system is greater than normal. In the normal cases about 10 per cent of aortic blood flows into the coronary arterial system, but in patients with this malformation more than half the aortic blood may be diverted into the abnormal coronary artery. Most of this blood is not

directed into the myocardial capillaries, which constitute a zone of high resistance; instead, the major part of the blood is directed through the low-resistance channel, the anomalous communication. In addition, blood from the other coronary artery may be diverted through intercoronary anastomoses to the fistula. Thus, for example, in a case in which the right coronary artery connects anomalously with a cardiac chamber, the myocardium in the distribution of the left coronary artery may be ischemic from the diversion of blood toward the fistula.

Hemodynamic studies may readily permit the identification of the site of anomalous communication of a coronary artery with a right-sided chamber or the pulmonary trunk. They do not, however, reveal the anatomic basis for the shunt. Ruptured aneurysm of an aortic sinus, ventricular septal defect with aortic insufficiency, and aorticopulmonary communications—all these may yield findings similar to those observed in anomalous communication of a coronary artery with a right-sided cardiac chamber or the pulmonary trunk. Precise anatomic diagnosis requires selective aortography.

In Mozen's case,<sup>37</sup> in which the fistula communicated with the left atrium, the findings of catheterization of the right side of the heart were normal.

#### Roentgenographic Features

Roentgenographic examination of the thorax revealed moderate to severe cardiomegaly

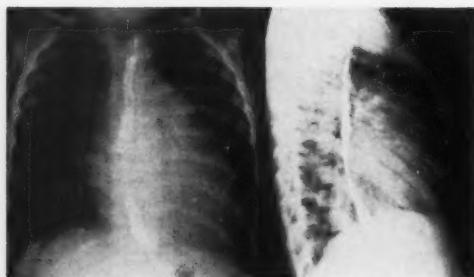


Figure 2

*Case II. Thoracic roentgenograms. Left. Postero-anterior view. Right. Lateral view. In this case, the left coronary artery communicated anomalously with the right ventricular chamber. There are cardiomegaly, prominence of pulmonary arterial shadow, prominence of the aortic knob, and a suggestion of increased vascularity of the lungs. The left-to-right shunt measured 1.3 liters per minute per square meter of body surface area.*

in four cases (cases II, III, IV, and V, fig. 2). In the two remaining patients (cases I and VI), the size of the cardiac shadow was within normal limits. The pulmonary arterial segment was prominent in all cases, and the peripheral pulmonary arterial vasculature was moderately to markedly increased in all but one patient (case VI).

The aortic knob was prominent in all cases. From conventional films, one cannot distinguish the findings in this anomaly from those in other conditions in which left-to-right shunts and continuous machinery murmurs occur. In general, the roentgenographic appearance seems to depend on the hemodynamic alterations of the particular case.

Gasul and associates,<sup>16</sup> summarized the roentgenographic findings in 28 cases, including five of their own. In nine cases these were considered to be normal; in the other 19 cases, the common findings were enlargement of the left ventricle, dilatation of the pulmonary arterial segment, and prominence of the pulmonary vasculature.

Although retrograde aortography by means of an injection through a radial artery is a time-honored procedure for diagnosing patent ductus arteriosus, it is very often unsatisfactory for diagnosing lesions originating in the

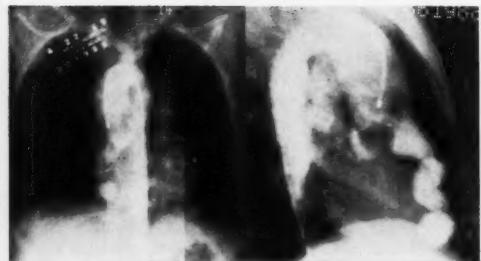


Figure 3

*Case I. Selective aortograms made into the origin of the aorta. A wide tortuous right coronary artery courses along the surface of the heart. Left. Anteroposterior view. Right. Lateral view. Although the enlarged right coronary artery communicated anomalously with the right ventricular chamber, the radiopaque material is not identified in the right ventricle at this stage of examination.*

ascending aorta; these lesions include: anomalous communication of a coronary artery with a cardiac chamber or the pulmonary trunk, aortopulmonary septal defect, persistent truncus arteriosus, or ruptured aneurysm of an aortic sinus of Valsalva.

In most cases, opaque material reaching the arch of the aorta by way of a retrograde injection follows the flow of blood in the aorta distally into the descending aorta. Thus the entire ascending aorta, including its sinuses, is usually not satisfactorily visualized by this method. This phenomenon was well demonstrated in one of our patients when two retrograde aortograms failed to demonstrate the anomalous termination of a coronary artery.

We prefer to use selective aortography, a procedure in which radiopaque material is injected into the base of the aorta through a catheter. Selective aortography was performed in three cases (cases I, II, and III): In case I, the aorta was shown to be dilated and densely opacified (fig. 3). The right coronary artery, which was unusually dilated, measured approximately 1½ to 2 cm. in diameter. Multiple saccular aneurysms were seen along the course of the anomalous vessel. Sequential filling of the right ventricle and pulmonary artery was observed following demonstration of anomalous communication

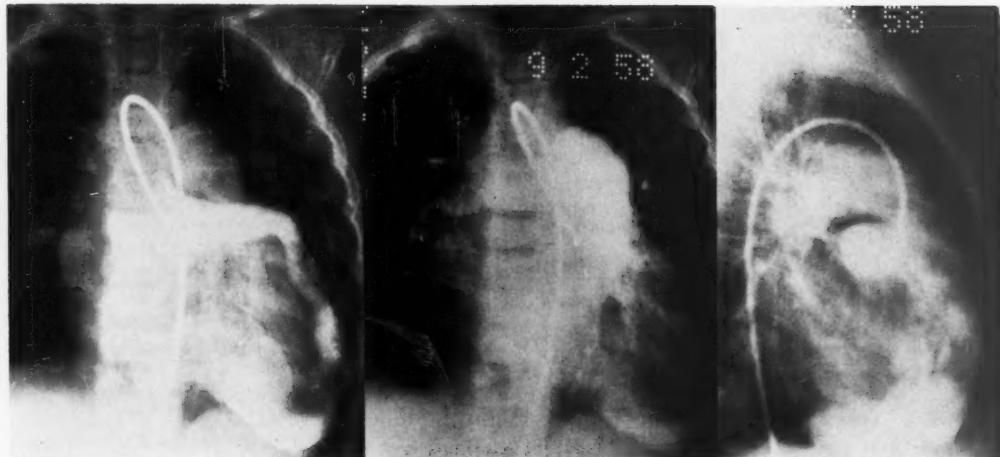


Figure 4

*Case II. Selective aortograms made into the origin of the aorta. Left. There is filling of a wide and tortuous left coronary artery as well as entrance of radiopaque material into the right ventricular cavity. Center. Anteroposterior view made 3 seconds after that shown in left and 11 seconds after injection; it reveals that the radiopaque material seen in the right ventricle has now passed into the pulmonary arteries. Right. Lateral view made at the time of exposure seen in center. Opacified material is observed in the pulmonary arteries and some is still present in the right ventricle.*

of the right coronary artery with the right ventricle.

In case II (fig. 4), a large, tortuous, irregularly dilated vessel arising above the left aortic sinus became apparent immediately after filling of the aortic sinuses. This channel progressed to the left and passed downward in the region of the anterior interventricular groove. Filling of this channel was followed by opacification of the right ventricular chamber and then of the pulmonary artery in normal sequence.

In case III, opacification of the left coronary artery was followed by filling of the main pulmonary artery, without opacification of the right ventricle.

#### Summary

Anatomic, clinical, hemodynamic, and roentgenographic findings in six patients with congenital communication of a coronary artery with a cardiac chamber or the pulmonary trunk are presented. The literature is reviewed.

A coronary artery may communicate anomalously with any of the cardiac chambers,

more commonly with those on the right side. In the six cases presented, the right coronary artery communicated with the right atrium in one case and with the right ventricle in another. The left coronary artery communicated with the right atrium and right ventricle in one case each, and with the pulmonary trunk in two cases.

The most striking feature observed clinically was a continuous murmur. If a continuous murmur is localized in an area atypical for patent ductus arteriosus, the diagnosis should be suspected. Conventional roentgenographic and electrocardiographic studies yielded no specific diagnostic features. The results of cardiac catheterization may reveal a left-to-right shunt, but they are diagnostically useful only when correlated with clinical findings.

The only precise method of demonstrating the abnormality is by means of selective aortography performed by injecting medium into the very origin of the aorta.

Cure is possible by surgical interruption of the fistulous tract.

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### On Permanent Patency of the Mouth of the Aorta, or Inadequacy of the Aortic Valves

By DOMINIC JOHN CORRIGAN, M.D.

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... From its singular and striking appearance, the name of *visible pulsation* is given to this beating of the arteries. It is accompanied with *bruit de soufflet* in the ascending aorta, carotids, and subelavians; and in the carotids and subelavians, where they can be examined by the finger, there is felt *fremissement*, or the peculiar rushing thrill, accompanying with *bruit de soufflet* each diastole of these vessels. These three signs are so intimately connected with the pathological causes of the disease, and arise so directly from the mechanical inadequacy of the valves, that they afford unerring indications of the nature of the disease.

## The Mechanism of Respiratory Variation in Splitting of the Second Heart Sound

By ROBERT F. CASTLE, M.D., AND KENNETH L. JONES, B.A.

MANY RECENT reports have stressed the importance of auscultatory analysis of the second heart sound as a part of routine cardiac evaluation. In spite of recognized changes in the second sound, which occur in a variety of disease states, there is still disagreement concerning the mechanism of normal respiratory splitting of the second sound.<sup>1, 2</sup>

Normal inspiratory splitting of the second heart sound was first described by Potain nearly a century ago.<sup>3</sup> It is only in the last decade, however, that this has become widely recognized as a physiologic finding in both adults<sup>4</sup> and children.<sup>5</sup> The first component of the second sound represents aortic valve closure and is transmitted over the entire precordium. It normally represents the only portion of the second sound heard at the apex and primary aortic area.<sup>6</sup> The second or pulmonic component of the second sound is best heard over the pulmonic area and is transmitted to a limited extent along the left sternal border, but not to the apex or primary aortic area. Thus, the optimum location for clinical and phonocardiographic study of physiologic splitting is over the pulmonic area.

That splitting of the second heart sound represents asynchronous closure of the aortic and pulmonary valves can be illustrated in several ways. Leatham<sup>7</sup> recorded the heart sounds simultaneously at the apex and pulmonic areas and clearly showed the coincidence of aortic closure with the first component of the second sound and pulmonic closure

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with the second. Taking into account the delay in carotid pulse transmission, Perloff and Harvey<sup>1</sup> demonstrated the synchronism of the first and second components of the second sound with the dicrotic notches of the carotid and pulmonary arterial pulses, respectively. Moreover, using intracardiac phonocardiographic technics, Rogers et al.<sup>8</sup> noted that the second component disappeared on removal of the pulmonary valve.

Normal respiratory variation is characterized by increased splitting in inspiration and approximation, or even fusion, of the two components during expiration. The usual explanation attributes inspiratory splitting solely to delayed pulmonic closure resulting from the increased stroke volume and lengthened ejection time of the right ventricle in this phase of respiration.<sup>1, 7, 9, 10</sup> This is a consequence of augmented right-sided filling secondary to the increase in negative intratho-

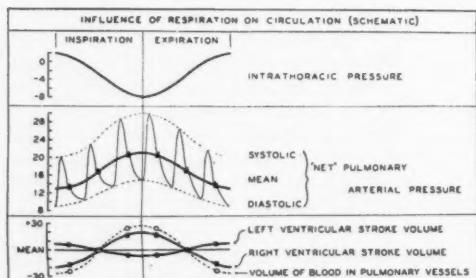


Figure 1

Diagram illustrating the influence of respiration on "net" pulmonary pressure, left and right ventricular stroke volumes, and the volume of blood in the pulmonary vessels. (Reproduced with permission of the publishers and the authors. Lauson, H. D., Bloomfield, R. A., and Cournand, A.: The influence of respiration on the circulation in man; with special reference to pressures in right auricle, right ventricle, femoral artery and peripheral veins. Am. J. Med. 1: 315, 1946.)

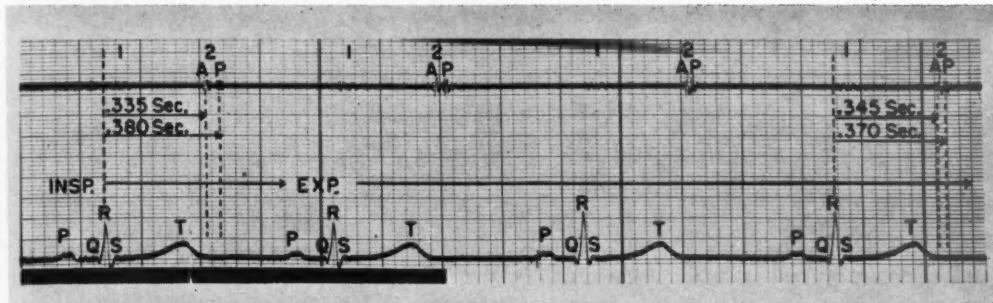


Figure 2

Representative phonocardiogram recorded over pulmonic area. Inspiratory and expiratory "R-A<sub>2</sub>" and "R-P<sub>2</sub>" values are shown. Individual measurements are shown to the nearest .005 second.

racic pressure accompanying inspiration. Leatham<sup>6</sup> has raised the possibility that a decrease in pulmonary artery pressure in inspiration may also be a factor in the delay in pulmonary valve closure.\* He believes, however, that the inspiratory increase in right ventricular output is more important. Recent work by Boyer and Chisholm<sup>2</sup> has indicated that expiratory lengthening of left ventricular systole also contributes to respiratory variation in second sound splitting. This view has not achieved general acceptance.

#### Methods and Materials

In order to study the mechanism of physiologic splitting, phonocardiograms were obtained from 80 normal children ranging in age from 5 to 15 years. Phonocardiograms were made on all patients while at rest and in the recumbent position. A Sanborn Twin-Beam apparatus with the standard dynamic microphone and a paper speed of 75 mm. per second was employed. Tracings were obtained over the pulmonic area with both the medium-sized (3.8 em.) bell and the black diaphragm. A simultaneous electrocardiogram, lead I, was recorded for timing purposes. All tracings were made as the subject respiration normally. An operator marked the tracing during each inspiratory portion of the respiratory cycle. Twelve of the 80 tracings were discarded because of a lack of clarity of one or both of the components of the

second sound. The 68 others were deemed satisfactory for analysis. In all but eight of these, the tracings obtained with the diaphragm end piece were analyzed.

With the tip of the R wave of the electrocardiogram as a constant point of reference, the time intervals between the R wave and the first and second components of the second sound were measured to the nearest .005 second (fig. 2). In each subject five cardiac cycles occurring at the peak of normal inspiration and five at the peak of normal expiration were measured. An average "R-A<sub>2</sub>" and "R-P<sub>2</sub>" interval was then obtained during normal inspiration and expiration. The contribution of the movement of each component of the second sound to respiratory splitting was then assessed.

With a paper speed of 75 mm. per second, one can measure individual intervals confidently only to the nearest .005 second (.375 mm.). For each subject five separate "R-A<sub>2</sub>" and "R-P<sub>2</sub>" measurements were averaged and then expressed to the nearest .001 second. In the text and figures all intervals expressed to the thousandths of a second are mean values and should not be interpreted as indicating that individual measurements of this accuracy can be attained with this method.

#### Results

The results of the analysis of these data are presented in figures 3 and 4 and show that a significant contribution to inspiratory splitting is made by earlier aortic valve closure. The movement of "A<sub>2</sub>" ranged from zero to .016 second, with an average of .007 second (S.D. = .004 second). Its movement contributed an average of 35 per cent (S.D. = 17 per cent) to the difference between the split-

\*This refers to a decrease in pulmonary artery pressure as measured against a constant baseline of atmospheric pressure. Effective ("net") pulmonary artery pressure or intravascular minus intrathoracic pressure increases during inspiration (fig. 1).

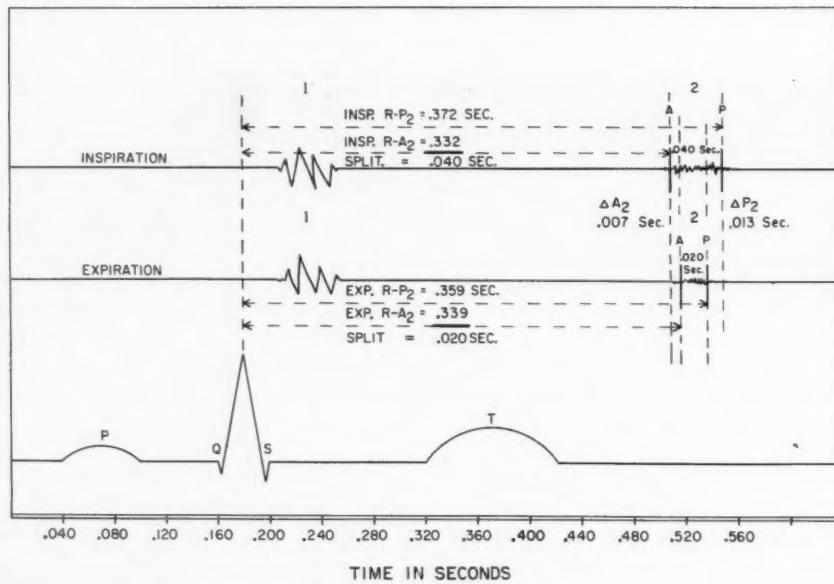


Figure 3

*Schematic representation of respiratory variation in splitting of the second sound showing the average measurements found in this series of normal children. Note the contribution of earlier aortic closure to inspiratory splitting.*

ting interval in inspiration and expiration (fig. 5). During normal respiration we found an average expiratory splitting of .020 second (S.D. = .008 second) with a range of from .009 to .039 second. In inspiration, splitting averaged .040 second (S.D. = .009 second) with a range of from .020 to .063 second.

#### Discussion

Our findings are in agreement with those of Boyer and Chisholm<sup>2</sup> in demonstrating a significant contribution of earlier aortic valve closure to inspiratory splitting of the second heart sound.

Lauson et al.,<sup>11</sup> Wiggers,<sup>12</sup> and Williams and Gropper<sup>13</sup> maintained that during inspiration an increase in pulmonary vascular capacity greater than that needed to accommodate the increased right heart output occurs. As a consequence, in inspiration pulmonary venous return to the left heart is decreased. This is associated with a transient fall in left-sided output (fig. 1) and an accompanying shortening of left ventricular ejection time

and hence slightly earlier closure of the aortic valve. On the other hand, as expiration begins, the pulmonary vasculature recoils,<sup>14</sup> thus increasing pulmonary venous return and augmenting left ventricular stroke volume (fig. 1) and ejection time.\*

Maximal splitting is noted at the peak of inspiration<sup>6, 15</sup> and corresponds to that phase of the respiratory cycle in which right ventricular stroke volume is greatest and left-sided output is relatively low (fig. 1).

\*Since this article was submitted for publication, Shafter (Am. J. Cardiol. 6: 1013, 1960) has reported on a study of the respiratory variation in splitting of the second sound. He suggested another explanation for the expiratory increase in left ventricular stroke volume, noting that there was some evidence that expiratory decrease in pulmonary vascular capacity did not occur. He pointed out that the augmented inspiratory right heart output requires a few seconds to reach the left heart. Thus, the apparent expiratory prolongation of left ventricular systole may be merely coincidental, reflecting instead the delayed effect of the augmented right heart output of the preceding inspiratory cycle.

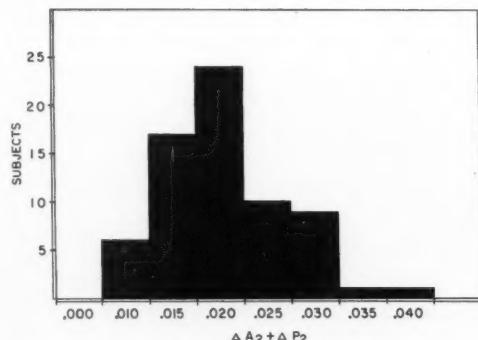


Figure 4

*Histogram showing variation in splitting interval with normal respiration. Note that in 51 of 68 subjects splitting variation ranged between .015 and .025 second.*

It is not unexpected that the aortic component of the second sound shows less movement than the pulmonic. Shuler et al.<sup>14</sup> and Dupee and Johnson<sup>16</sup> suggested that a three-fold inspiratory increase in right ventricular stroke volume may occur but that expiratory augmentation of left-sided output does not exceed 50 per cent. These large fluctuations, however, were noted under artificial experimental conditions. We believe that the data of Lauson et al., showing a 25 per cent inspiratory condition. We believe that the data of Lauson et al., showing a 25 per cent inspiratory increase in right ventricular stroke volume and a 7 per cent expiratory increase in left ventricular stroke volume, more closely reflect the situation in the human subject. The greater respiratory variation in right-sided volumes probably reflects the inherent greater distensibility of the right ventricle.<sup>17</sup> In addition, the large capillary volume of the lungs represents an effective buffering reservoir that tends to prevent sudden extreme changes in the pulmonary venous return.<sup>16</sup>

The distinction of pathologic from physiologic splitting of the second sound is not always simple. The auditory threshold for the clinical recognition of splitting approximates .02 second.<sup>18</sup> Thus, in many normal subjects the second sound is appreciated as split, although usually variably so, throughout the

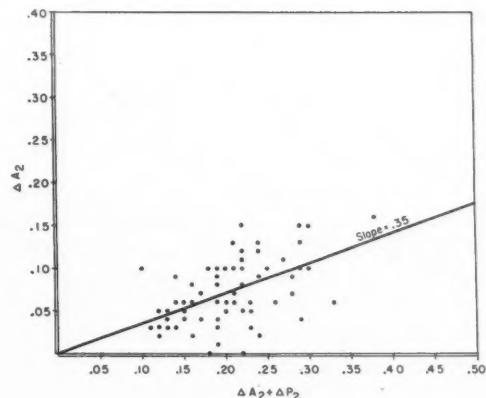


Figure 5

*Scatter diagram showing contribution of movement of aortic valve closure to total variation in splitting of second sound. The slope (.35) indicates an average contribution of 35 per cent.*

respiratory cycle. Moreover, a recent report has shown that some individuals exhibit a widely and nearly constantly split second sound while in the supine position.<sup>19</sup> When upright, however, these patients demonstrate the expected respiratory variation in splitting. These observations suggest that further phonocardiographic studies of a normal population with respect to positional changes in splitting of the second sound would be of value.

Analysis of the second sound is of importance in the evaluation of patients with a variety of congenital and acquired cardiac diseases. Changes include abnormally wide splitting on a "mechanical" basis (e.g., atrial septal defect, pulmonic stenosis, ventricular septal defect, mitral regurgitation) or on an "electrical" basis (complete right bundle-branch block).<sup>20</sup> On the other hand, abnormally narrow or reversed splitting also occurs. The "mechanical" type is noted in patent ductus arteriosus<sup>21</sup> and aortic stenosis<sup>20</sup> whereas the "electrical" variety is present in complete left bundle-branch block.<sup>20</sup>

A common clinical situation in which knowledge of normal splitting of the second sound is particularly useful is in distinguishing the patient with an atrial septal defect

from the patient with an innocent pulmonic ejection murmur. In the vast majority of patients with a hemodynamically significant atrial defect wide and constant splitting (.04 second or more) of the second heart sound is present.

#### Summary

This study of 68 normal children has shown that in the supine position and under conditions of normal respiration splitting of the second sound averages .02 second in expiration and .04 second in inspiration. Inspiratory increase in splitting of the second sound is the result not only of a delay in pulmonic valve closure but also of earlier aortic closure. Movement of the aortic component is responsible, on the average, for 35 per cent of the difference in splitting of the second sound in inspiration and expiration.

#### Acknowledgment

We are indebted to Mr. Robert L. Nicks, Superintendent, Methodist Home for Children, Raleigh, North Carolina, for allowing us to use children of the Home as subjects for this study. We also gratefully acknowledge the assistance of Dr. H. B. Wells, Department of Statistics, School of Public Health, University of North Carolina.

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# The Pulmonary Vessels in Incipient Left Ventricular Decompensation

## Radiologic Observations

By MORRIS SIMON, M.D.

THE APPEARANCE of clinical symptoms in a patient with an overloaded or weakened left ventricle generally provides the earliest evidence that the compensating cardiac mechanisms are no longer able to cope with normal physiologic demands.<sup>1</sup> If, however, clinical signs and symptoms appear only after a certain level or duration of left ventricular decompensation, then the asymptomatic, apparently compensated patients must include some in what might be termed "incipient" left ventricular decompensation.<sup>2, 3</sup> It might be important to recognize such patients as an intermediate group; they may readily regress into the symptomatic group, or alternatively the symptoms might be anticipated or even prevented by appropriate therapy.

The radiologist may contribute in this area by detecting objective evidence of left ventricular decompensation before clinical symptoms have appeared. Characteristic changes in the pulmonary vascular pattern are seen on the plain chest roentgenogram.

### Radiologic Findings

#### Normal

Before consideration of these changes, it is helpful to review some of the normal radiologic features of the pulmonary vasculature (fig. 1).<sup>4-6</sup> The pulmonary arteries diverge from the hilus well above the point at which the veins converge on the left atrium, at least 3 or 4 cm. in the adult. Thus, in the mid and lower zones of the lungs the direction of a

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vessel helps identify it as artery or vein. In the upper zones, the arteries and veins run in the same direction but the vein lies lateral to its corresponding artery. The main upper-lobe veins may be seen to cross the main pulmonary artery at an angle inconsistent with entry into it. In the normal person the upper-lobe vessels, both veins and arteries, are substantially smaller than those in the lower lobe. This normal disparity is due to the conical shape of the lung with the lower-lobe vessels subserving a greater volume of tissue.

This normal vascular pattern is encountered in some patients with left ventricular disease, but almost invariably without symptoms of left ventricular decompensation (fig. 2). They are thus well compensated by both clinical and radiologic criteria.

#### Pattern of Overt Left Ventricular Decompensation

Practically all our patients with clinical evidence of left ventricular decompensation observed in the last 5 years have shown, with greater or lesser clarity, a characteristic change in the pulmonary vascular pattern (fig. 3).<sup>4</sup> In the upper zones the pulmonary veins undergo marked dilatation and the pulmonary arteries also dilate slightly. In the lower zones, paradoxically, the veins and arteries do not dilate and in many cases show clear-cut evidence of narrowing. They may be difficult to identify, contrasting markedly with their normal ready visibility and good caliber. An absolute change of caliber of single vessels is usually clearly detectable, but the disparity between the dilated upper vessels and relatively narrowed lower-zone vessels is more striking. It should not be forgotten that normally the upper-zone vessels

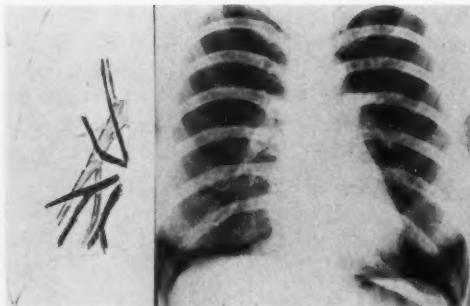


Figure 1

*Normal pulmonary vascular pattern. The upper-lobe vessels are substantially smaller than those in the lower lobe. In the diagram the left hilar vessels are drawn with the arteries light and the veins dark.*

are actually smaller than those in the lower zones.

In the more severely decompensated cases, vascular detail may be partly obscured by pulmonary edema. This may be predominantly interstitial, causing a diffuse faint haziness at the lung bases occasionally with the appearance of septal lines of Kerley,<sup>3, 7-10</sup> or may be true alveolar edema with scattered irregular areas of consolidation progressing to the classic "butterfly" pattern of consolidation.<sup>5, 11-13</sup> Pleural effusions may occur with accentuation of the interlobar fissures. The cardiac silhouette may show a rapid enlargement. Such patients are invariably symptomatic, however, and the diagnosis of left ventricular failure is seldom in doubt (fig. 4).<sup>1</sup>

#### *Pattern of Incipient Left Ventricular Failure*

Many patients without symptoms of left ventricular decompensation, though frequently with a history, previous clinical documentation, or radiologic evidence of left ventricular disease, have the typical vascular pattern of left ventricular decompensation. The roentgen examination shows dilated upper-lobe vessels and narrowed lower-lobe vessels.

Are these changes due to "incipient" left ventricular decompensation? Several findings obtained favor this possibility: 1. A prolonged circulation time has been demonstrated in

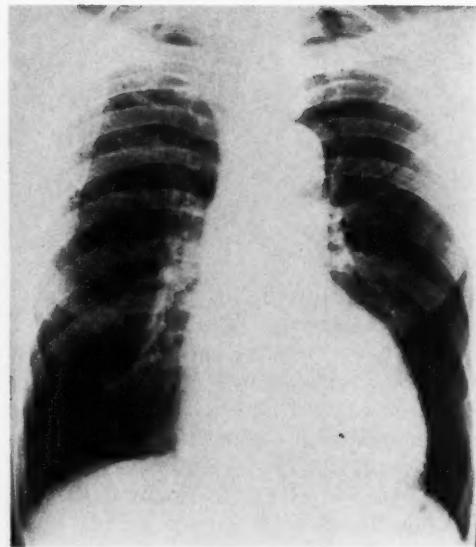


Figure 2

*Normal pulmonary vascular pattern in patient with fully compensated aortic stenosis and substantial left ventricular enlargement.*

some cases, a few returning to normal values after treatment. 2. Rapid and significant weight loss in response to diuretic therapy has occurred in a number of such symptomless patients. 3. In a few patients effectively treated by diuretics, the pulmonary vascular pattern has returned to normal. 4. The concept has sometimes been tested in less fortunate manner. The typical observations on pre-operative films have been ignored on occasion, and two such cases developed pulmonary edema during surgery. Occasionally, patients presenting with pneumonia or pulmonary embolism may have an unsuspected component of left ventricular failure, possibly precipitated by the infection or embolism. In these cases the radiologist may observe the dilatation of the upper-lobe vessels and constriction of the lower-lobe vessels, in addition to the focal lesion, and is thus able to amplify the diagnosis, leading to better therapy. In one case with lobar pneumonia, the consolidation responded poorly for approximately 10 days until the decompensation was treated, after which resolution was dramatic (fig. 5).

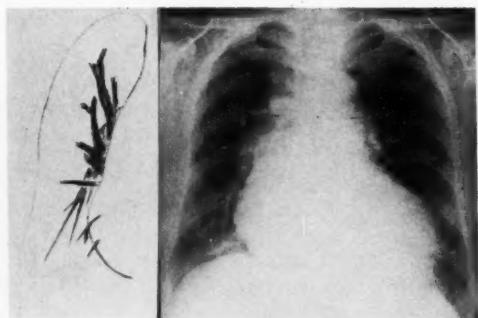


Figure 3

Pulmonary vascular pattern in left ventricular failure. There is a marked disparity between the dilated upper-lobe vessels and the relatively narrowed lower-zone vessels.

The vascular pattern of left ventricular decompensation, whether clinically overt or incipient, is sometimes a transient phenomenon, returning rapidly to normal with effective treatment. More commonly, however, the vascular changes are persistent. It is possible that such individuals may be incapable of regaining complete physiologic compensation and remain in a chronic state of "incipient" decompensation.

The widely held concept that the venous overdistention in left ventricular failure is a passive manifestation of damming back of blood, or elevation of the pulmonary venous pressure, fails to account for the decreased caliber of the vessel in the lower zones. In 1956, the writer proposed an alternative hypothesis to explain a similar pulmonary vascular pattern in patients with mitral stenosis<sup>4, 14-16</sup> and suggested its applicability in left ventricular failure (fig. 6):

1. In the erect position of man, since the veins of the upper and lower lobes communicate freely via the left atrium, the pressure in the lower-lobe veins is normally substantially greater than in the upper-lobe veins because of the difference of hydrostatic level between them.

2. A significant rise in pulmonary venous pressure, almost invariable in mitral stenosis, is probably also an early effect of left ventricular decompensation.<sup>2, 14</sup>

3. There is reasonable evidence to postulate

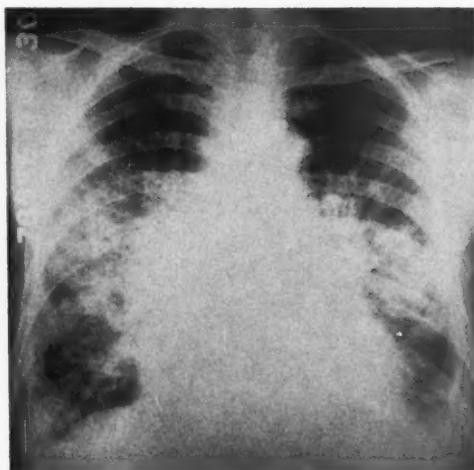


Figure 4

Pulmonary edema in overt left ventricular decompensation. The lower-lobe vessels are obscured.



Figure 5

Left ventricular decompensation associated with pneumonia of the middle lobe.

a localized segmental reflex, initiated by a rise of pulmonary venous pressure above a critical level, that results in constriction of the veins and arteries in that particular lung segment.

4. In left ventricular failure the rising venous pressure reaches this critical level in the lower zones long before it does in the upper zones. Vasoconstriction thus occurs initially in the lower zones, reducing the circulating volume through them.

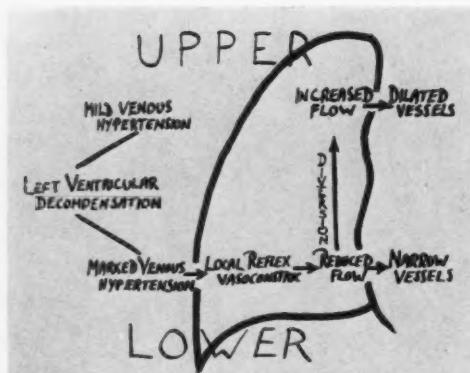


Figure 6  
Hemodynamic hypothesis to account for the vascular pattern of left ventricular decompensation.

5. Cardiac output is maintained by diversion of blood through the upper zones. Thus, the vascular dilatation in the upper lobes represents increased blood flow and is not due to "damming back" or "congestion" of the blood.

It is tempting to speculate on the mechanism of orthopnea on the basis of this hypothesis. In the upright position the vasoconstrictive phenomena are confined to the lower zones and cardiac output is maintained by increased flow through the upper zones. In the horizontal position, the pressures in the veins of the upper and lower zones are equalized at intermediate values. In the less severe cases, the pressures might still be generally below the critical level and the horizontal position would thus be tolerated. In more severe cases the critical level would be widely exceeded and generalized reflex vasoconstriction would occur with rapid restriction of blood flow through the lungs. The resulting oxygen lack would in turn stimulate respiratory efforts.

Much of the evidence in support of the vasoconstrictive reflex hypothesis is derived from prior studies on cases of mitral stenosis in which the elevation of pulmonary venous pressure is slowly progressive and catheterization data were available.<sup>4, 14, 15</sup> From these studies it would appear that the critical venous pressure at which vasoconstriction occurs is of the order of 10 to 15 mm. of mercury (normal, 0 to 5 mm.).<sup>17</sup>

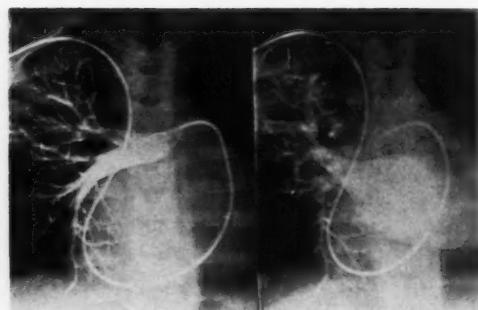


Figure 7  
Pulmonary angiography in cardiac decompensation due to primary myocardial disease. Left. Arterial phase. There is slight dilatation of the upper-lobe arteries and marked constriction of the lower-lobe arteries. A capillary blush appears early in the upper lobe. Right. Venous phase. The upper-lobe veins are greatly dilated and show early filling. The lower-lobe veins are barely visible. The contrast medium lingers in the constricted lower-zone arteries, indicating retardation of flow. (By courtesy of Drs. E. D. Neuhauser, Children's Medical Center, Boston and the Journal of the Canadian Association of Radiologists.<sup>18</sup>)

Angiograms (fig. 7) in a 7-year-old boy in congestive cardiac failure due to primary myocardial disease, with elevated wedge pressure at 25 mm. of mercury, provide convincing support for the hypothesis. In the early film (fig. 7, left) there is slight dilatation of the upper-zone arteries and marked constriction of the lower-zone arteries. In the later film (fig. 7, right) there is gross dilatation of the upper-zone veins whereas the lower-zone veins are barely visualized and are undoubtedly constricted.

Flow through the lower zone is greatly retarded while the relative rate of flow through the upper zone is greatly increased. Thus, the contrast medium outlines the great upper-lobe veins and passes into the left atrium via the superior orifice, long before it has even negotiated the constricted lower-zone arteries. This supports the concept that the vascular dilatation in the upper zone is due to diversion of blood from the lower zone,

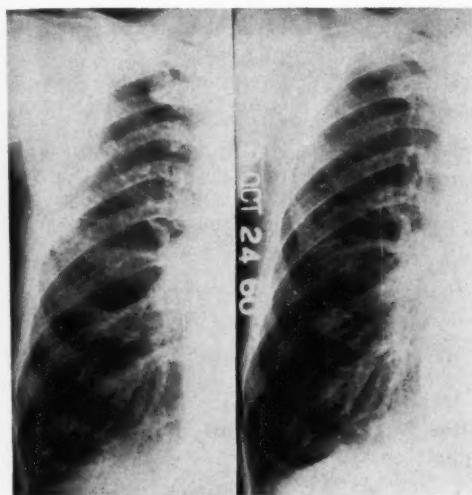


Figure 8

Severe emphysema without failure (left) and complicated by left ventricular failure (right). The lower-zone vessels have dilated as well as the upper-zone vessels, representing an exception to the general rule.

probably with locally increased flow, and not due directly to a damming back of blood.

An exception to the typical vessel pattern in left ventricular failure occurs in patients with severe pulmonary emphysema or chronic bronchitis (fig. 8). In these cases both the lower-lobe vessels and the upper-lobe vessels appear to become dilated, simulating the generalized dilatation of pulmonary vessels seen in cases of left-to-right shunt with increased flow through the lungs. The explanation is uncertain and may be related to shunting of blood through overdeveloped bronchial collateral vessels or failure of the postulated reflex vasoconstriction mechanism.

The pattern of vascular dilatation in the upper zones and constriction in the lower zones characterizes other causes of pulmonary venous hypertension, e.g., mitral stenosis, left atrial tumors, and some cases of pericardial effusion.<sup>4, 14-18</sup>

It may also sometimes be seen in cases of basal bronchiectasis or basal emphysema without pulmonary venous hypertension in which pulmonary circulation in the lower lobes is

reduced by the local parenchymal disease, and for this reason blood becomes diverted through the upper lobe vessels.<sup>4</sup>

#### Conclusion

An awareness of the typical pulmonary vascular pattern associated with left ventricular decompensation may facilitate early diagnosis of this condition, not infrequently before symptoms have appeared. In such cases the symptoms may be anticipated or prevented by appropriate treatment.

#### Summary

Dilatation of the pulmonary vessels of the upper zones and constriction of the vessels of the lower zones characterizes left ventricular decompensation.

These changes may precede the onset of clinical symptoms providing a useful warning sign of impending danger.

A hypothesis is suggested to account for the non-uniform vessel pattern.

An exception is noted in patients with severe emphysema or bronchitis in whom generalized dilatation may occur.

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### The Idea of Chemical Transmission of Nervous Impulse

Now I have to turn to the best known of my scientific achievements, the establishment in 1921 of the chemical theory of the transmission of the nervous impulse. Until 1921 it was generally assumed that transmission was due to the direct spreading of the electrical wave accompanying the propagated nervous impulse from the nerve terminal to the effector organ. Since the character of that potential is everywhere the same, such an assumption would not explain the well-known fact that the stimulation of certain nerves increases the function of one organ and decreases the function of another. A different mode of transmission had, therefore, to be considered.

As far back as 1903, I discussed with Walter M. Fletcher from Cambridge, England, then an associate in Marburg, the fact that certain drugs mimic the augmentary as well as the inhibitory effects of the stimulation of sympathetic and/or parasympathetic nerves on their effector organs. During this discussion, the idea occurred to me that the terminals of those nerves might contain chemicals, that stimulation might liberate them from the nerve terminals, and that these chemicals might in turn transmit the nervous impulse to their respective effector organs. At that time I did not see a way to prove the correctness of this hunch, and it entirely slipped my conscious memory until it emerged again in 1920.

The night before Easter Sunday of that year I awoke, turned on the light, and jotted down a few notes on a tiny slip of thin paper. Then I fell asleep again. It occurred to me at six o'clock in the morning that during the night I had written down something most important, but I was unable to decipher the scrawl. The next night at three o'clock, the idea returned. It was the design of an experiment to determine whether or not the hypothesis of chemical transmission that I had uttered seventeen years ago was correct. I got up immediately, went to the laboratory, and performed a simple experiment on a frog heart according to the nocturnal design. I have to describe briefly this experiment since its results became the foundation of the theory of chemical transmission of the nervous impulse.

The hearts of two frogs were isolated, the first with its nerves, the second without. Both hearts were attached to Straub cannulas filled with a little Ringer solution. The vagus nerve of the first heart was stimulated for a few minutes. Then the Ringer solution that had been in the first heart during the stimulation of the vagus was transferred to the second heart. It slowed and its beats diminished just as if its vagus had been stimulated. Similarly, when the accelerated nerve was stimulated and the Ringer from this period transferred, the second heart speeded up and its beats increased. These results unequivocally proved that the nerves do not influence the heart directly but liberate from their terminals specific chemical substances which, in their turn, cause the well-known modifications of the function of the heart characteristic of the stimulation of its nerves.—OTTO LOEWI. An Autobiographic Sketch. *Perspectives in Biology and Medicine* **4**: 16, 1960.

# The Genesis of Hyponatremia Associated with Marked Overhydration and Water Intoxication

By JAMES M. STORMONT, M.D., AND CHRISTINE WATERHOUSE, M.D.

THE PRESENCE of severe hyponatremia, not associated with salt depletion, is now well known in a wide variety of disease states. In nearly all situations in which it has been studied, low serum sodium concentrations have been accompanied by an increase in body water and thus the term "dilutional hyponatremia" has been used. In spite of the well-known dilutional factor, certain studies have indicated changes of serum cation not accounted for by fluid and electrolyte balances.<sup>1-4</sup> The hyponatremia in these states may be due not only to dilution but also to an internal reduction of osmotic activity. The present study was designed to evaluate the role of overhydration in the latter process. We have used daily injections of Pitressin Tannate in Oil\* to induce overhydration and marked hyponatremia while maintaining strict balance conditions. This approach has allowed further study of the mechanism involved in the genesis of severe hyponatremia, water intoxication, and the diuretic escape from Pitressin.

## Methods

Thirteen balance studies were conducted in 10 patients hospitalized on the metabolism ward of the Strong Memorial Hospital (table 1). Each patient was in a separate, temperature-controlled room. A constant diet was administered and this was analyzed twice during each study. Constituents of each diet are recorded in table 1. All excreta were collected quantitatively and stool collections were separated into periods with carmine markers. Data were collected for water, sodium, potassium, chloride, and nitrogen balance. The patients were weighed at the end of each 24-hour period and a fasting blood sample was obtained without exposure to air. Close observation by specially trained

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\*Parke, Davis & Company, Detroit, Michigan.

nurses and physicians throughout the day and night provided an accurate record of symptomatology.<sup>†</sup>

Following suitable adjustment on a constant diet, a control period of 3 days was begun, which was followed by a treatment period during which 1 or 2 units of Pitressin were injected twice daily. In several instances, overhydration was reduced with infusion of 10 to 12.5 per cent mannitol, and Pitressin was continued. A control period after Pitressin was utilized whenever possible. In some studies other drugs were administered throughout both control and Pitressin periods to minimize symptomatology. This included atropine in studies 4 and 5, paraldehyde in study 1, Gelusil in studies 7 and 8, and either chloral hydrate or Nembutal to all.

Sodium and potassium were analyzed on an internal-standard flame photometer. Chloride was analyzed with automatic titration<sup>5</sup> with a Cotlove chloridimeter,<sup>5</sup> and nitrogen was analyzed by the maero-Kjeldahl method. Osmolality was analyzed by freezing-point depression, and calcium was determined by an established method.<sup>6</sup> Potassium balance was corrected for nitrogen balance (2.7 mEq. K/Gm./N). An insensible loss of 5 mEq./day for sodium, potassium, and chloride was assumed although no visible sweat was noted.

Changes in chloride space were calculated by standard methods.<sup>2</sup> An initial chloride space of 20 per cent body weight was assumed. Changes in total body water were calculated with the Newburgh modifications<sup>7</sup> of Lavet's formula for fat balance.<sup>8</sup> Previous studies have indicated a close correlation between changes in body water determined by this method and the deuterium dilution method.<sup>9, 10</sup> Initial total body water was assumed to be 60 per cent of body weight. With use of the observed serum sodium concentration, sodium and

<sup>†</sup>For the purposes of graphic representation, symptoms of water intoxication were graded as follows: Grade I, mild (lethargy, drowsiness, malaise, fatigue, nervousness, bloated feeling, weakness, headaches). Grade II, moderate (anorexia, epigastric "hunger pains," nausea, frequent stools, abdominal cramps, tightness of the chest, minimal vomiting). Grade III, severe (haggard appearance, diarrhea, delirium, marked nausea with vomiting). Severe coma or convulsions were not observed. There was no marked change in blood pressure in any subject.

Table 1  
Clinical Data and Dietary Intake

Study	Patient	Age, sex, diagnosis	Initial weight Kg.	Na (mEq.)	K (mEq.)	Daily intake N (Gm.)	H <sub>2</sub> O (liters)	Calories
Group I								
1	A.G.	63, M, chronic alcoholism and recent pneumonia	59	20	83	12.4	3.2	2570
2	I.F.	46, F, chronic alcoholism and recent pneumonia	53	47	68	9.3	2.2	1390
3	M.S.	47, F, chronic alcoholism	56	86	90	8.2	2.0	1800
4	D.C.	50, F, fractured hip	77	95	87	11.5	1.9	1860
5	D.C.	50, F, fractured hip	74	16	126	11.5	1.6	1860
Group II								
6	O.H.	54, M, chronic alcoholism, gout, coin lesion—lung	64	187	110	17.0	2.8	3220
7a	F.F.	54, M, reformed alcoholic, cirrhosis, emphysema	57	11	61	7.3	1.9	1370
7b	F.F.	54, M, reformed alcoholic, cirrhosis, emphysema	59	11	61	7.3	2.5	1370
8a	A.F.	40, F, obesity, bilateral pulmonary hilar adenopathy	121	16	48	6.8	1.9	715
8b	A.F.	40, F, obesity, bilateral pulmonary hilar adenopathy	121	16	48	6.8	2.6	715
Group III								
9	I.C.	54, M, chronic bronchiectasis	45	77	90	8.8	2.6	2020
10	S.M.	30, M, chronic alcoholism and recent pneumonia	73	102	112	17.5	3.3	2770
11	T.C.	35, M, chronic alcoholism and recent pneumonia	78	177	97	16.4	2.2	2115

potassium balance, and calculated total body water, values were estimated for total body osmotically active cation, change in osmotically active cation unaccounted for by electrolyte balance, and changes in serum sodium concentration predicted by water and electrolyte balance.\* Isotonicity

\*Body cation concentration was assumed to be the serum sodium concentration corrected for Donnan and protein factors plus 10 mEq./L. Total body fluid osmotically active cation (TBC) was calculated as the product of calculated total body water (TBW) and cation concentration. The change in osmotically active cation unaccounted for by balance ( $\Delta uOAC$ ) was calculated as follows:  $\Delta uOAC = TBC_2 - TBC_1 - b(Na+K)$ , where  $b(Na+K) =$  the sum of sodium and potassium balance and  $TBC_1$  and  $TBC_2 =$  the total body cation at the beginning and end of a period. Predicted serum cation concentration at the end of a period was calculated as follows:  $[TBC_1 + b(Na+K)]/TBW_2$ . The change in serum sodium concentration unaccounted for by balance = Observed  $\Delta Na$  - Predicted  $\Delta Na = \Delta uNa$ . Per cent change in serum sodium concentration unaccounted for by balance =  $\% \Delta uNa = \text{Observed} \Delta Na - \text{predicted} \Delta Na \times 100 / \text{observed} \Delta Na$ . The absolute value for observed  $\Delta Na$  was used as divisor. (See appendix for sample calculations.)

among body fluid compartments was assumed.<sup>12, 13</sup> The use of serum sodium as an arbitrary point for calculating cation balance has been of value in evaluating osmotic changes, and errors inherent in this method have been discussed.<sup>1, 10, 13</sup> Furthermore, a linear relation has been demonstrated between corrected serum sodium concentration and serum osmolality (corrected for blood urea nitrogen and blood sugar).<sup>14</sup> In our studies, there was a close relationship between serum sodium and serum osmolality. When total fall in sodium concentration was contrasted with total fall in serum osmolality divided by two, a discrepancy of 2 mEq./L. or less was noted in all but one instance, in which the difference was 4 mEq./L.

Renal concentrating ability was estimated by the U/P osmolar ratio determined on 24-hour urine specimens, and by net solute-free water reabsorption ( $T^*H_2O = Cosm - V$ ).  $T^*H_2O$  was determined following infusions of 10 to 12.5 per cent mannitol at a rate of 10 to 20 ml. per minute. No catheter was used. Subjects were fasting and received distilled water 20 ml./Kg. by mouth 1 hour prior to the infusion except as noted in table 4. After urine flow greater than 5 ml. per minute was established with mannitol, aqueous Pitressin, 100 mU, was administered through the intravenous tubing and 100 mU were added to the infusion,

which ran 30 to 45 minutes (i.e., 1 to 2 mU/Kg./hour).  $T^{\circ}\text{H}_2\text{O}$  was determined from plasma and urine samples obtained during the infusions and corrected to 1.73M.<sup>2</sup> body surface area.

### Results

#### Clinical and Balance Data

The studies have been divided into three groups based on the response to Pitressin: Group I, marked fluid retention with severe water intoxication; group II, initial antidiuresis, with escape from Pitressin effect; and group III, minimal fluid retention (tables 1 and 2).

#### Group I

Balance data were obtained in five studies on four patients (table 2 and figs. 1 and 2). Studies 4 and 5 on subject D.C. were carried out as a continuous balance experiment. After 9 days of Pitressin administration on a 95-mEq. sodium diet (study 4), the sodium intake was reduced to 16 mEq./day and Pitressin was stopped. Following equilibration on this intake, Pitressin was again administered for 17 days (study 5).

A prompt antidiuretic effect occurred in all studies with urine volumes 27 to 48 per cent of the control values and osmotic U/P ratios of 2.5 to 4.1. Fluid retention occurred at a rate of 0.4 to 2 liters a day and mild symptoms of water intoxication began on the second to the fourth days. A slight escape from Pitressin antidiuresis was evident after the third or fourth day with increasing sodium and chloride excretion, urine volumes 58 to 79 per cent of control values and osmotic U/P ratios of 2.1 to 3.8 (table 2). As progressive overhydration and hyponatremia continued, symptoms became marked and potassium excretion increased without a corresponding fall in sodium excretion. Severe water intoxication developed in all instances, forcing discontinuation of Pitressin after 6 to 12 days.

There are several points of interest in the balance data of these patients. 1. The Pitressin-induced loss of 359 mEq. of sodium and 90 mEq. of potassium during study 4 (subject D.C.) did not affect the development of a

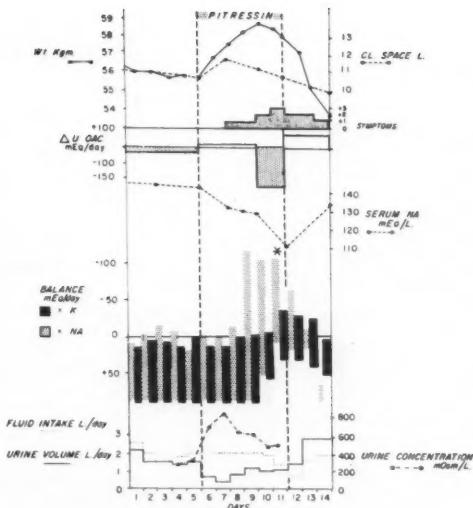


Figure 1

*Balance study 3 (M.S.) showing rapid development of severe water intoxication with marked cation losses unaccounted for by balance. (\*) Denotes emesis which was analyzed and included in data. Balance data are graphically represented by plotting intake downward from zero line. The bars then represent output and the area above the zero line indicates negative balance.*

typical, although delayed, response to Pitressin during study 5 with further electrolyte loss. 2. Mannitol-induced diuresis in study 5 (fig. 2) rapidly corrected the overhydration from Pitressin. The patient was much improved symptomatically by this procedure. The prompt return of marked antidiuresis with Pitressin is also clearly seen. 3. Although the increase in urinary potassium was marked in some patients of this group, the total potassium loss was never excessive (the cumulative potassium balance varied from 0 to -90 mEq.). In most instances the potassium loss began before the patient's intake of food was limited by severe nausea or anorexia.

#### Group II

In this group there were five studies in three patients (table 2 and figs. 3 and 4). Studies 7a and 7b were a continuous experiment and are shown together in figure 3. The only change in the experimental design of these two studies was an increased fluid intake

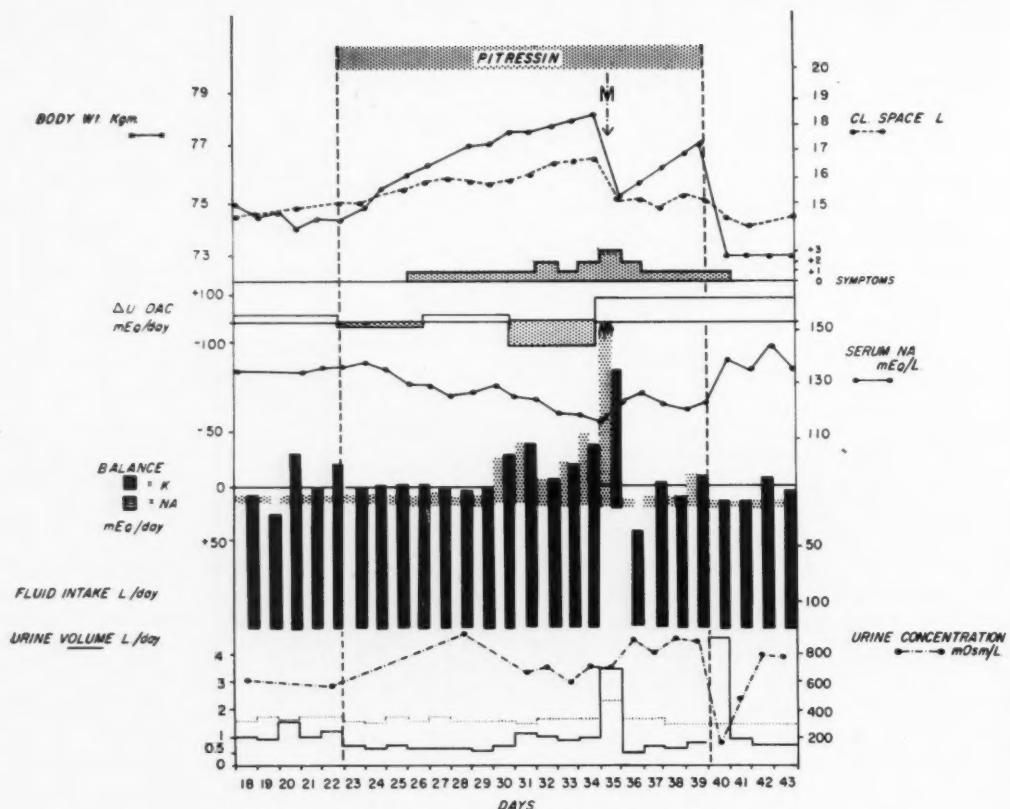


Figure 2

Balance study 5 (D.C.) showing the gradual development of severe water intoxication in a moderately salt depleted subject. (M) denotes hypertonic mannitol infusion which was followed by loss of overhydration, gain in cation unaccounted for by balance ( $\Delta uOAC$ ), and improvement in symptoms. On day 35, the sodium balance was  $-212 \text{ mEq}$ .

in study 7b. Studies 8a and 8b were also carried out continuously on the same patient (A.F., fig. 4), and again the major change in the two studies was increased fluid intake.

The antidiuretic response was less marked than in group I with urine volumes 44 to 63 per cent of control (table 2). Overhydration occurred initially but fluid retention rapidly became less marked or absent. The escape from Pitressin antidiuresis was more pronounced than in group I, with urine volumes 85 to 87 per cent of control and osmotic U/P ratios 2.0 or less. Loss of sodium and chloride occurred at a rate similar to group I. Potassium loss was less marked than in

group I and in some instances was inversely related to sodium loss (fig. 4). Symptoms of water intoxication were less prominent than in group I.

With increased fluid intake (studies 7b and 8b, figs. 3 and 4) and continuous Pitressin administration, fluid retention again became marked and peripheral edema occurred. (Both subjects had a history of peripheral edema.) Intermittent diuresis with urine volumes in excess of control and osmotic U/P ratios equal to or less than 1.0 limited continued gain in total body water. This diuresis was not associated with an increase in urinary sodium and chloride. Total cumulative

Table 2

## Fluid and Electrolyte Changes

Group I	Days on Pitressin	Urine volume*			U/P mOsm		Gain in total body water			
		Control	Minimum Liters/day	Maximum Liters/day	Maximum	Minimum	Maximum Liters	Maximum rate L./day*	Mean rate L./day	Mean balance Na mEq./day
Study	1	7	2.4	0.7	1.3		8.3	2.0	0.9	-28 0
	2	7	1.8	0.6	0.9	2.5 2.4	5.5	1.3	0.8	-27 -2
	3	6	1.7	0.6	1.1	3.0 2.1	3.2	1.1	0.4	-59 -3
	4	9	1.7	0.7	0.9	4.1 3.8	2.5	0.4	0.2	-44 -10
	5	12	1.2	0.6	1.0	3.6 2.8	5.1	0.6	0.4	-8 -4
Group II	6	7	2.1	1.3	1.8	2.8 2.0	5.9	1.0	0.8	-51 +1
	7a	7	1.3	0.5	1.1	2.7 1.0	4.9‡	0.7	0.4	-14 -3
	b	10	†	1.2	2.0	1.4 0.4		0.6	0.1	-20 -4
	8a	8	1.0	0.6	0.9	2.5 1.2	6.1‡	0.5	0.4	-9 -7
	b	14	†	1.0	1.8	1.4 0.8		0.5	0.2	-14 +3
Group III	9	8	2.2	1.7	2.8	1.4 0.7	1.7	0.4	0.1	-13 -2
	10	9	2.3	1.8	2.5	2.0 1.0	2.0	0.3	0.2	+14 -3
	11	8	1.4	1.2	2.0	2.9 1.0	2.1	0.6	0.1	-8 -5

\*Figures represent mean values for 3-day periods.

†Continuous study—no control.

‡Over-all maximum gain for studies a and b.

gain in body water was similar in groups I and II (table 2).

A good antidiuretic response to Pitressin was again observed following mannitol-induced diuresis in study 8b (fig. 4).

### Group III

Three patients. There was a minimal antidiuretic effect with more rapid increase in urine volume, and osmotic U/P ratios of 1.0 or less (table 2). Overhydration and hyponatremia were less than in groups I and II. Sodium and chloride losses were less marked and usually inversely related to potassium losses.

### Changes in Electrolyte Concentration and Distribution

Serum sodium concentration fell to the range of 100 to 113 mEq./L. in group I after 6 to 12 days of Pitressin administration and increasing overhydration (figs. 1 and 2 and table 3). In group II, with similar degrees of overhydration, serum sodium remained above 115 mEq./L. (figs. 3 and 4 and table 3). There was a rough correlation between serum sodium concentration and degree of

symptomatology. Mild symptoms were seen with serum sodium concentration in the range of 120 to 130 mEq./L. Moderate symptoms were associated with serum sodium concentrations of 114 to 120 mEq./L., and severe symptoms occurred in all subjects with serum sodium concentrations below 114 mEq./L. Serum potassium concentrations varied less than 1 mEq./L. throughout and there was no consistent change in blood urea nitrogen or hematocrit even with marked sodium losses. Serum carbon dioxide content fell slightly from control values of 26 to 32 mM/L. to values of 22 to 24 mM/L. during severe hyponatremia.

Changes in serum sodium concentration, which could not be accounted for by cation and fluid balance ( $\Delta uNa$ ), occurred in all studies (table 3). Although variable changes in  $\Delta uNa$  occurred with the development of mild to moderate hyponatremia, greater than 50 per cent of the fall in serum sodium concentration was unaccounted for at the lower serum sodium concentrations (table 3 and fig. 5). Changes in total body fluid os-

Table 3  
Changes in Serum Sodium Concentration Not Accounted for by Balance

Study	Days*	Final serum Na mEq./L.	(A)	(B)	ΔuNa Difference (A) - (B) mEq.	% ΔuNa (A) - (B) (A) × 100 per cent	Symptoms†
			Observed change mEq.	Predicted change mEq.			
1	4	114	-20.7	-25.6	+ 4.9	+ 24	2+
	3	108	- 5.4	0	- 5.4	-100	3+
2	4	117	-20.4	-18.5	- 1.9	- 9	1+
	3	100	-17.5	- 8.5	- 9.0	- 53	3+
3	4	126	-15.1	-16.5	+ 1.4	+ 7	1+
	2	111	-15.5	- 3.9	-11.6	- 75	3+
4	6	121	-17.9	-12.2	- 5.7	- 32	1+
	3	113	- 8.2	- 2.6	- 5.6	- 68	3+
	5†	132	+19.2	+ 7.5	+11.7	+ 61	0
5	7	128	- 7.0	-10.2	+ 3.2	+ 46	1+
	2	122	- 5.7	- 3.8	- 1.9	- 33	1+
	3	112	-10.2	- 5.1	- 5.1	- 50	3+
	4†	134	+17.6	+ 9.8	+ 7.8	+ 44	0
6	2	121	-23.6	-21.4	- 2.2	- 9	0
	5	117	- 4.3	- 4.8	+ 0.5	+ 10	2+
7	7	127	-11.8	-12.5	+ 0.7	+ 6	1+
	3	118	-- 9.1	- 8.3	- 0.8	- 9	2+
8	4	133	- 7.3	-12.4	+ 5.1	+ 70	0
	4	125	- 8.3	-10.5	+ 2.2	+ 26	1+
	4	117	- 7.4	- 7.4	0	0	2+
	9	128	-14.7	-11.1	- 3.6	- 24	1+
10	5	136	+ 7.3	+ 5.6	+ 1.7	+ 23	0
	3	133	- 4.9	- 6.0	+ 1.1	+ 22	0
11	3	137	+ 4.5	+ 1.6	+ 2.9	+ 64	0
		138					

\*Data represent sequential periods of Pitressin administration except for studies 7 to 11 where data represent only periods in which the observed change in serum sodium was at least 1 mEq./day, and for recovery periods (†) where Pitressin was not given.

†See footnote page 191.

motically active cation were directly related to  $\Delta$  uNa and reflect the same mechanism. Maximal  $\Delta$  uOAC losses (greater than 80 mEq./day in studies 1-5) also occurred with severe hyponatremia and water intoxication (figs. 1-4). During recovery from severe hyponatremia, induced either by intravenous mannitol administration or by discontinuance of Pitressin, a gain in cation unaccounted for by balance (both  $\Delta$  uNa and  $\Delta$  uOAC) was observed (figs. 1-5 and table 3).

Additional data taken from the reports of others<sup>4, 15, 16</sup> fall in the same range as data from this study (fig. 5). When this data are represented as a per cent of the observed fall

in serum sodium,\* a good correlation is evident (fig. 5). A curve fitted to the data in figure 5 by the method of least squares, has a formula of  $Y = 0.26X + 123.1$  if  $Y$  = final serum sodium concentration and  $X$  = %  $\Delta$  serum sodium unaccounted for by balance. Correlation coefficient is 0.90 with a  $p$  value of less than 0.01. In all subjects with severe symptoms, over 50 per cent of the fall in serum sodium was unaccounted for by balance and serum sodium concentrations were below 114 mEq./L., regardless of the rate of change of observed serum sodium concentra-

\*See footnote page 192.

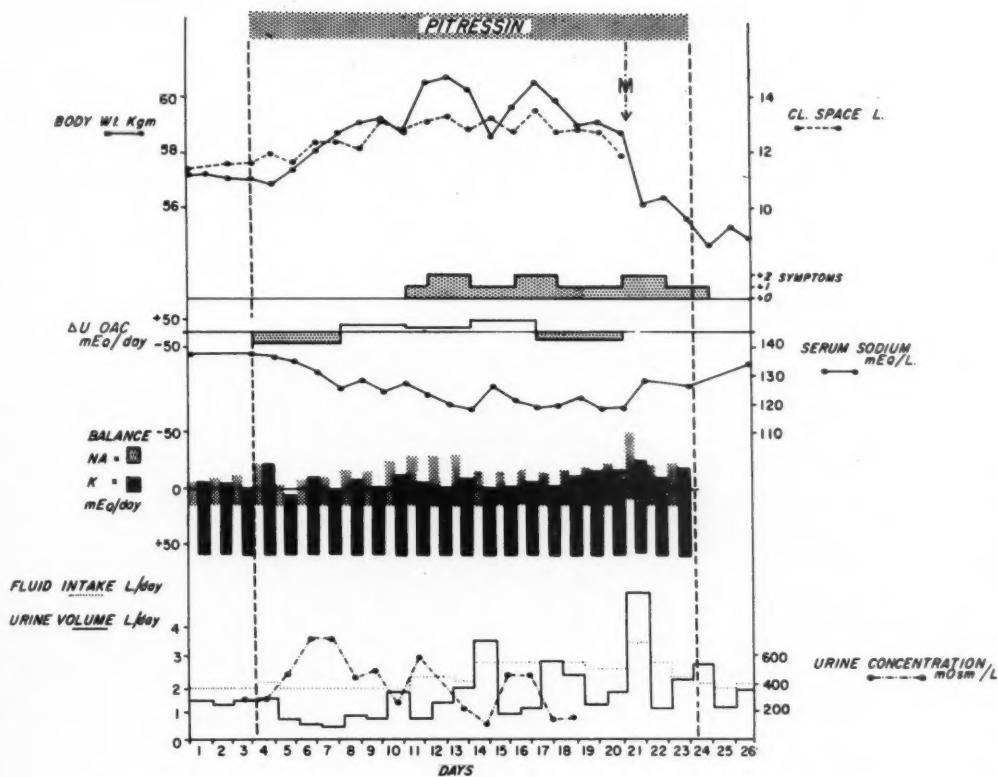


Figure 3

Balance study 7 (F.F.) showing prolonged overhydration with intermittent episodes of hypotonic diuresis, and failure to develop severe water intoxication even with increased fluid intake. (M) denotes hypertonic mannitol infusion. Period 7a includes days 4 to 10, while 7b includes days 11 to 20.

tion, the rate of fluid retention or the total gain in total body water (figs. 1, 2, and 5 and table 3).

#### Renal Response to Pitressin

Progressive overhydration in groups II and III was limited by intermittent periods of isotonic or hypotonic diuresis. This increase in urine volume occurred spontaneously in response to Pitressin-induced overhydration, and was not associated with increased total solute excretion (fig. 6), calcium excretion or potassium loss (tables 2 and 4). In addition, there was no correlation with variations in fluid, nitrogen, sodium, or potassium intake (table 1). This diuresis began in group III

subjects as early as 2 days following the start of daily Pitressin injections, at a time when there had been no marked gain in total body water (table 2).

Studies of renal function during infusion of hypertonic mannitol and Pitressin revealed a correlation between increase in total body water and maximum ability to concentrate the urine in the presence of an osmotic load (table 4). Following spontaneous diuresis with loss of overhydration (studies 9 and 11) the response to hypertonic mannitol and Pitressin more closely approximated the normal response (table 4 and fig. 7). A marked diuretic effect and weight loss of 1 to 3 Kg.

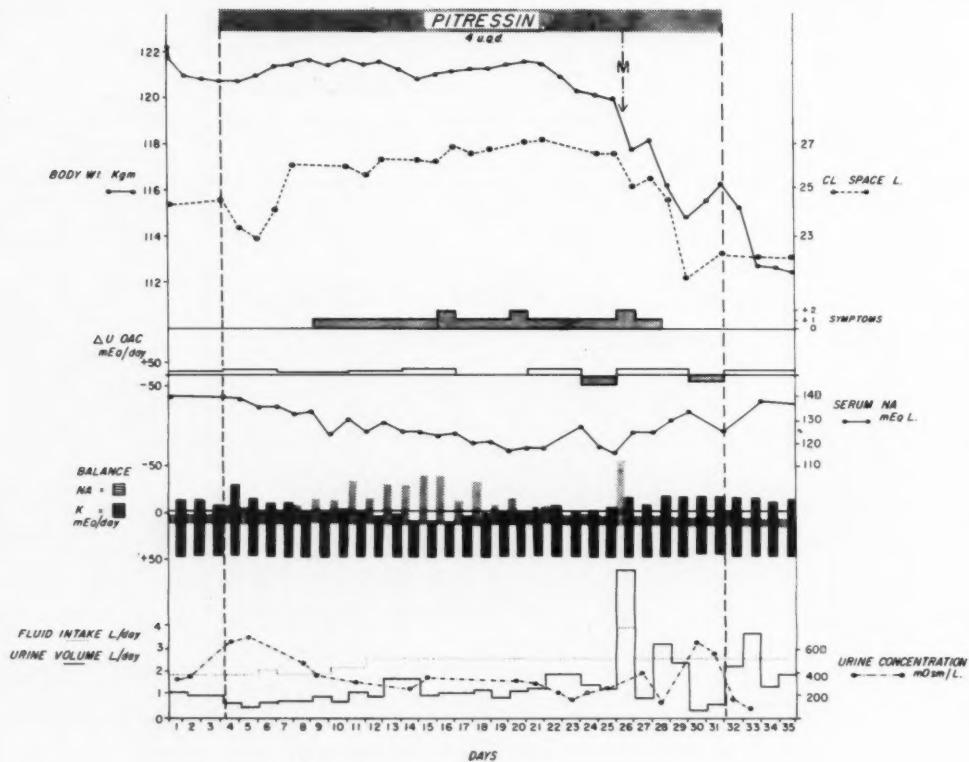


Figure 4

Balance study 8 (A.F.) showing prolonged overhydration in subject receiving a low-calorie diet with escape from antidiuretic effect and failure to develop symptoms of severe water intoxication. (M) denotes hypertonic mannitol infusion. Period 8a includes days 4 to 11, and 8b includes days 12 to 25.

in 24 hours was observed following mannitol infusion in those patients with overhydration and decreased  $T^cH_2O$ , in spite of continued daily Pitressin administration. This defect in renal concentration during osmotic loading could not be directly correlated with the spontaneous low solute diuresis which limited overhydration in groups II and III.

Estimation of one aspect of adrenal function through measurement of urine hydroxy steroid excretion revealed a consistent difference between group I subjects in whom antidiuresis was maintained, as contrasted with groups II and III subjects in whom antidiuresis was less marked and of limited duration. In groups II and III a fall in 24-hour urine total hydroxyecorticoid excretion

occurred during the first 3 to 5 days of Pitressin administration followed by rapid return to control values at 7 to 9 days. In group I, the fall in corticoid excretion was more pronounced and the return to normal less rapid (fig. 8).

#### Discussion

Hyponatremia associated with alterations in total body osmotically active cation unaccounted for by balance has been postulated in certain disease states.<sup>1-4, 17-19</sup> Our studies demonstrate a marked decrease in osmotically active cation, which was unaccounted for by balance and an observed fall in serum sodium concentration greater than predicted. This was induced by severe overhydration and was

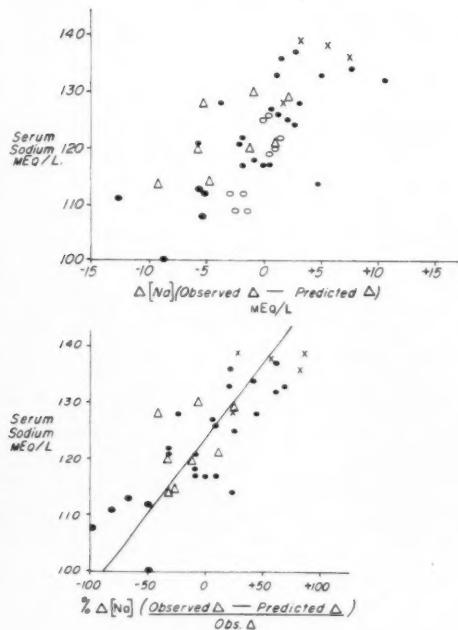


Figure 5

Derived data from all balance studies (●) showing changes in serum sodium concentration unaccounted for by fluid and electrolyte balance as related to final serum sodium concentration (table 3). Data from the literature are included as follows: Δ (16), X (4), and 0 (15). The lower graph depicts this data as a per cent of observed change, and a curve fitted to this data has a formula  $Y=0.26 \times +123.1$ .

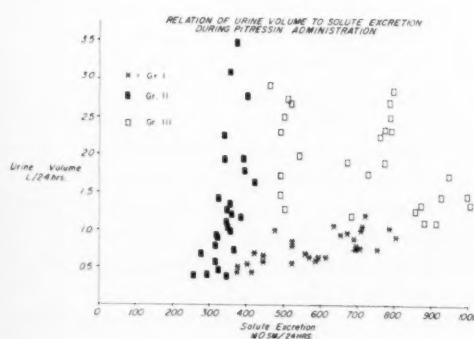


Figure 6

Solution excretion and urine volume for subjects in groups I, II, and III.

Table 4  
Variations in Renal Concentrating Ability During Prolonged Pitressin Administration

A. Receiving daily Pitressin	Prior to infusion			During infusion*			Post infusion		
	Serum Na mEq/L	Urine Na mEq/day	K <sup>+</sup> mEq/day	No intravenous Pitressin			Urine volume ml/min.	Solute output mOsm/min.	T <sub>1.73M<sup>2</sup></sub> ml/min.
				Δ Total body water liters†	Urine volume ml/min.	Solute output mOsm/min.			
Study—5	112	320	-142	+4.6	15.2	+0.88	24.2	5.8	-0.8
7	118	9	-63	+3.5	16.5	4.3	+0.6	20.0	4.4
8	116	2.5	-405	-20	25.1	4.7	-4.9	30.0	6.5
9	129	38	307	+22	12.9	3.2	-0.8	20.8	5.7
9†	136	41	284	+3	6.5	2.2	+2.1	10.7	3.4
11†	141	136	435	-50	9.3	3.1	+1.5	15.4	5.0
B. No daily Pitressin								+3.2	+0.2
Study—5	134	1	175		5.4	2.7	+4.08		
7	138	9	275		7.0	2.4	+1.5	9.5	3.8
11	139	146	361		9.1	3.5	+2.1	13.9	5.4
								+4.3	+4.3
									151
									-0.3

\*Infusion of 12.5 per cent mannitol. Intravenous Pitressin=100mU rapidly i.v. + 1.2 mU/Kg/hour added to the infusion of mannitol. Subjects prehydrated.

†Cumulative balance for each subject.

§After spontaneous diuresis with loss of overhydration.

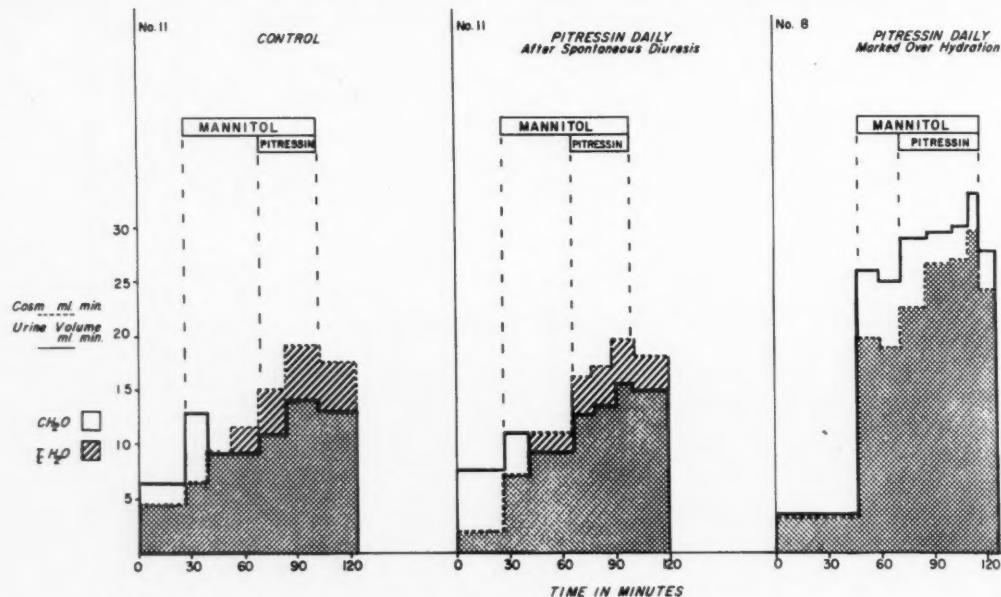


Figure 7

Clearance studies during infusion of hypertonic mannitol and aqueous Pitressin in patients A.F., (no. 8) and T.C. (no. 11). Water load was given orally 1 hour prior to mannitol infusion.

not associated with an active disease state. It was readily reversible, either by discontinuation of Pitressin and allowing water diuresis to occur, or by inducing osmotic diuresis with intravenous hypertonic mannitol in the presence of continuing Pitressin administration. Correction of overhydration was associated with a gain of osmotically active cation unaccounted for by balance, a rise in serum sodium concentration greater than predicted, and a loss of symptomatology within 24 to 72 hours. Unaccountable cation changes were most evident at serum sodium concentrations below 114 mEq./L. In this range, over 50 per cent of the fall in serum sodium concentration was unaccounted for by fluid and cation balance, and severe symptoms of water intoxication were noted. At higher serum sodium concentrations, the unaccounted for changes in cation were less prominent, and daily variations were evident with cation gains often balancing cation losses.

While certain studies lend support to the

hypothesis that cellular hypotonicity may be a factor in the development of hyponatremia,<sup>1-4, 17-19</sup> other studies have failed to show a loss of cation other than that unaccounted for by balance.<sup>15, 20-22</sup> The present study suggests that such losses may become evident only with severe degrees of fluid retention. Previous attempts to demonstrate unaccounted for loss of cation resulting from overhydration in man, have dealt with serum sodium concentrations in the range of 118 to 130 mEq./L., a range where undetected cation loss was minimal or variable in our studies. There are certain difficulties in applying data from azotemic or hyperglycemic dogs to the clinical situation; nevertheless, data of Wynn<sup>15</sup> reveal values that fall close to the values obtained in our data (fig. 5).

Loss of cation unaccounted for by balance may represent either an undetected external loss or an internal alteration in metabolism resulting in loss of osmotic activity. Our studies would not detect losses from the body

of hydrogen, magnesium, or amino acids that are capable of acting as cations (lysine, arginine). Although we cannot exclude an undetected cation loss of this type, the rapid gain in unaccounted for cation with correction of overhydration would suggest that this was not a major factor. Reversible reduction of osmotic activity could be accomplished by cation binding in mucopolysaccharides, proteins, weak acids, or bone, and this would not necessitate an osmotic gradient between cells and extracellular fluid. The observation<sup>14</sup> that serum sodium concentration is proportional to the sum of exchangeable sodium and potassium divided by total body water even at low serum sodium concentrations, as well as other studies,<sup>11, 12</sup> suggests that there is no *sustained* osmotic gradient between cells and extracellular fluid. Our data suggest that with severe overhydration, isotonicity is maintained not only by fluid shifts, but also by reduction in total number of active cellular osmols. This mechanism could act as a homeostatic compensation tending to maintain cell volume.

Severe symptoms of water intoxication occurred in all studies in group I, and were associated with marked overhydration, negative sodium and potassium balance, and a fall in serum sodium concentration below 114 mEq./L. The failure to develop severe symptoms in group II during an equivalent degree of Pitressin-induced overhydration (table 2) was correlated with (1) intermittent episodes of increased urine volume without increased total solute output (figs. 3, 4, and 6) so that maximum gain in total body water was accomplished more slowly, and only after an increased fluid intake; (2) less fall in serum sodium with relatively close agreement of predicted and observed values (table 3 and fig. 5); and (3) less fall in total urine hydroxycorticoid excretion with more rapid return to or beyond control levels (fig. 8).

These studies indicate that certain patients receiving daily Pitressin injection may develop a failure to form concentrated urine. This is manifest both in response to simple

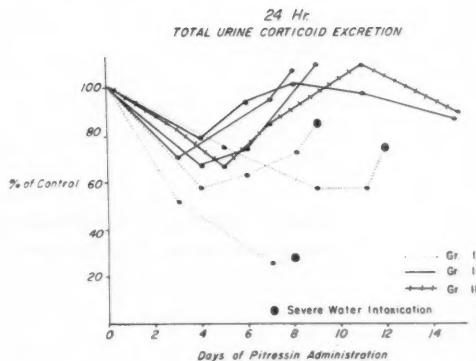


Figure 8

Urine hydroxycorticoid excretion indicating more pronounced fall in three studies of group I relative to three studies of group II and one of group III.

overhydration (continuous water load) or to intravenous mannitol (osmotic load). The spontaneous development of isotonic (or even hypotonic) urine was not directly correlated with either the degree or duration of overhydration. These data confirm in part earlier observations in animals<sup>23, 24</sup> and man.<sup>16, 25, 26</sup> Decreased permeability of the renal tubular cells to water may be responsible for this spontaneous escape from Pitressin effect.<sup>16</sup> In contrast, the inability to concentrate maximally during an osmotic load appeared to be related to the degree of overhydration and was corrected following dehydration. Previous studies have demonstrated that overhydration may affect renal concentrating ability.<sup>27-29</sup> This correlation with changes in body water and the lack of correlation with the spontaneous diuresis suggests that decreased solute concentration in the renal medullary interstitial fluid may be an etiologic factor. Osmotic mechanisms in urine concentration and dilution have been recently reviewed<sup>30</sup> and will not be further discussed here.

Escape from Pitressin-induced antidiuresis associated with prolonged overhydration may act as a self-regulatory mechanism during abnormal states to limit excessive fluid retention and consequent water intoxication. It is possible that the concentration defect observed in certain patients with cirrhosis<sup>31</sup>

and other states associated with overhydration and hyponatremia<sup>8</sup> may be related to a similar phenomenon.

The present data would suggest that certain clinical states associated with fluid retention, severe hyponatremia, and water intoxication may be benefited by fluid restriction, osmotic diuresis, or steroid therapy. Unfortunately one or all of these measures may be contraindicated in overhydration associated with severe renal, cardiac, infectious, or brain disease.

### Summary

Prolonged overhydration and hyponatremia have been produced in 10 patients with use of Pitressin Tannate in Oil. Balance studies have shown that in patients who developed moderate hyponatremia, the drop in serum sodium could be explained by water retention. In patients who developed severe water intoxication, the very low levels of serum sodium (100-114 mEq./L.) could not be entirely accounted for by changes in salt and water balance.

Certain patients failed to develop severe water intoxication although an equivalent degree of overhydration was achieved. In these subjects, further overhydration was limited by intermittent episodes of low solute diuresis. This diuretic escape from Pitressin effect has been evaluated by measurement of U/P osmolar ratio on 24-hour urine specimens as well as T<sup>3</sup>H<sub>2</sub>O during hypertonic mannitol infusion. Defects in both aspects of renal concentration were observed, although they were not necessarily coexistent.

### Appendix

#### Sample Calculations

Study 2: Control 3 days, Pitressin 7 days, control 4 days.

#### Balance Data—Days 8 and 9

1. I. L.=insensible weight loss=weight in—weight out— $\Delta$ body weight=5590-3480=—340=2450 or 1225 Gm. q.d.
2. Fb=fat burned=I. L.—(2.18C + 12.26 UN)=  

$$\frac{3.93}{1225-(2.18 \times 149 + 12.26 \times 8.0)=204 \text{ Gm.}}$$
3.  $\Delta$ BF=△body fat=fat in — fat burned =  

$$\frac{3.93}{\text{q.d.}}$$

- 61—204=—143 Gm. q.d.=—286 Gm./2 days.
- $\Delta$ BP=△body protein=6.25 $\times$ 0.08=—.5 q.d.=—1 Gm./2 days.
4.  $\Delta$ TBW=△total body water=△body weight— $\Delta$ BF —  $\Delta$ BP=—340—(—286)—(—1)=—53 ml.=—0.05L.
5.  $TBW_1$ =total body water, initial=36.97.
- $TBW_2$ =total body water, final=36.92.
6. Initial serum sodium=117.2, corrected=119.7= [Na]<sub>1</sub>.
- Final serum sodium=107.6, corrected=109.9= [Na]<sub>2</sub>.
7. b(Na + K)=balance of sodium + potassium (corrected)=—172 mEq.
8.  $TBC_1$ =total body cation, initial= $TBW_1 \times ([Na]_1 + 10)$   
 $TBC_1=36.97 \times 129.7=4795 \text{ mEq.}$
- $TBC_2$ =final  $TBC=26.92 \times 119.9=4427 \text{ mEq.}$
9.  $\Delta uOAC=TBC_2 - TBC_1 - b(Na + K)= -196 \text{ mEq. or } 98 \text{ mEq. q.d.}$
10.  $[Na]_{pr}$ =predicted serum sodium concentration =  $[cation]_{pr} - 10 = (TBC_1 + b(Na + K)) / TBW_2 - 10 = \frac{4795 + (-172)}{36.92} - 10 = 115.2$  mEq.

11.  $\Delta [Na]_{pr}=119.7 - 115.2= -4.5 \text{ mEq/L.}$
- $\Delta [Na]_{ob}$ =observed change in serum sodium concentration=119.7—109.9=9.8 mEq.
12.  $\% \Delta uNa = \frac{\Delta [Na]_{ob} - \Delta [Na]_{pr}}{\Delta [Na]_{ob}} = \frac{-9.8 - (-4.5)}{-9.8} = -54\% = \text{per cent of change in serum sodium concentration unaccounted for by balance.}$

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## Sequelae of Left Ventricular Puncture with Angiocardiography

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THE LEFT VENTRICLE may be catheterized from the aorta,<sup>1-3</sup> from the left atrium by several routes,<sup>4-6</sup> or may be directly punctured. Since 1958 we have performed left ventricular puncture in 142 cases for pressure measurements and contrast injection. It is the aim of this paper to report our complications together with a review of those reported by others.

### Material and Methods

Our cases were 6 months to 56 years old, most between 30 and 40 years of age. The diagnoses are presented in table 1. The main indications for the procedure were aortic stenosis or mitral insufficiency, and our results with it were recently reported.<sup>7-9</sup>

The investigation is performed with the patient in the fasting state, under penicillin prophylaxis with blood and necessary equipment for cardiac resuscitation in readiness. In adult patients general anesthesia is not used, only premedication with morphine and scopolamine. The puncture is performed by a thoracic surgeon in the presence of a cardiologist, an anesthesiologist, and a radiologist. We use the intercostal method of Brock et al.<sup>10, 11</sup> We only wish to stress that the needle used has a blunt end and a sharp mandrin. When the ventricular cavity is reached, the needle is locked by a screw so that it cannot be introduced farther. An electrocardiogram is continually monitored on a two-beam cathode-ray oscilloscope. The pressure curves from the left ventricle and a peripheral artery are recorded. The ventricular curve must be free and undamped up to the moment of contrast injection and during slight changes in the position of the needle, in order to avoid intramyocardial deposition of contrast medium. With an automatic pressure syringe we then inject 1 to 1.2 ml. per Kg. of body weight of 76 per cent Urograffin, with a speed of about

30 ml. per second. The pressures are again checked after the contrast injection. During the injection phase and immediately afterwards compression of both common carotid arteries is performed to diminish the flow of the dye to the brain. In order to avoid alteration in the position of the needle during this time, the patient is ordered to hold his breath. A chest radiogram of the frontal projection in the supine position is taken shortly afterwards; if this is unaltered, the patient is transported to the postoperative ward. For the next 20 hours he is closely supervised with frequent and regular observations of pulse and blood pressure. Then a chest radiogram in frontal and lateral projections in the sitting position and an electrocardiogram are taken and the patient is brought back to the medical ward. As a rule the patient leaves the hospital a few days later.

### Results

We have performed 142 left ventricular punctures on 137 patients. In four cases the puncture was unsuccessful. In one of these cases the needle caused very frequent ventricular premature beats. In two other cases the needle entered the right ventricle. No contrast material was injected, and the surgeon deemed it inadvisable to perform more punctures. In the fourth case ventricular fibrillation occurred, which necessitated cardiac resuscitation.

Of the remaining 138 punctures no injection was possible for technical reasons in four cases and in one case because of coagulation in the needle. There remained 133 successful punctures in 131 cases.

In most instances only slight or no pain was experienced during and immediately after the puncture. When the needle passed through the ventricular wall extrasystoles of ventricular origin were seen in practically all cases. When the needle end was free in the ventricular cavity, the pre-existing rhythm was restored as a rule. In one case

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Table 1

*The Diagnoses in 137 Cases Submitted to Left Ventricular Puncture*

Diagnoses	Number of cases
Aortic stenosis	16
Aortic insufficiency	6
Aortic stenosis and aortic insufficiency	13
Mitral stenosis	3
Mitral insufficiency	13
Mitral stenosis and insufficiency	17
Combined aortic and mitral	63
Ventricular septal defect	3
No valvular disease	3

A few of these cases were combined with other disorders, such as tricuspid stenosis and insufficiency, aortic coarctation, atrial septal defect, corrected transposition,<sup>14</sup> and extreme poststenotic aortic dilatation, due to proved cystic medial neerosis in one case.

a short run of supraventricular tachycardia (175 per minute) was registered during the puncture but soon subsided and contrast injection could be performed.

During and immediately after the angiography some events were regularly seen (table 2). During the injection 5 to 10 ventricular extrasystoles occurred, probably released by the contrast jet hitting the opposite endocardium. Preceded in some cases by asystole or bradycardia, a moderate tachycardia followed, accompanied by a blood pressure fall of varying degree. After the angiography some patients complained of headache, which was in no case severe, and a few experienced nausea.

A slight rise in temperature (37.5 to 38 C.) was noted in 74 cases during the first 24 hours. In 15 other cases the temperature exceeded 38 C., generally associated with minor or major complications. Four cases had pleuritic pain localized to the site of puncture for more than 24 hours without proved cause. One of them, however, had a pericardial friction rub.

The complications may be divided into minor and major groups.

#### Minor Complications

This group (table 3) includes those who experienced no or only slight inconvenience

Table 2

*Changes in Heart Rhythm and Rate during Left Ventricular Angiocardiography\**

Phase of investigation	Sinus	Atrial fibrillation	✓ES†	Asystole‡	Bradycardia	Tachycardia
Before injection	79	43	25	—	7	27
During injection and x-ray exposure	12	11	99	1	2	93
X-ray exposure (continued)	75	35	22	12	22	30
After x-ray exposure	79	43	5	—	2	80

\*Findings in 122 cases, in which full information was available.

†vES = (repeated) ventricular extrasystoles.

‡Asystole = cardiac standstill lasting more than 2 seconds.

and did not require special therapeutic measures.

1. Hemopericardium. There may always be some leakage of blood into the pericardium. Twenty-seven cases were operated upon within 2 months and in four cases the surgeon noted "enlarged amounts" of blood in the pericardial sac. None of these cases had clinical signs of cardiac tamponade, and the operation was performed 8, 8, 11, and 30 days after the puncture.

2. Pneumothorax was observed in eight cases and caused no subjective discomfort. The diagnosis was established by chest radiogram in seven cases and only by angiogram in one (being substernal). In the other seven cases it was left-sided and in all but one of minimal size. In this last case it was three fingers broad.

In the case diagnosed by angiography and in another a loud extra heart sound with varying position in the heart cycle was heard for several days. In the first patient it was audible only during inspiration and was so loud as to be heard at some distance, and by the patient himself. In the second case it was less loud, present in all phases of respiration and was followed some days later by a typical pericardial friction rub. The electrocardiogram and radiograms were normal. These phe-

Table 3  
*Minor Complications in 142 Left Ventricular Punctures*

Complication	Number
Hemopericardium without tamponade	4*
Pneumothorax	8
Pleural effusion	15
Parenchymatous pulmonary changes	8
Delayed onset of arrhythmia	2

\*This is a minimum number, representing only those found at surgery.

nomena were probably due to the presence of a small amount of air between the pericardium and the pleura, causing a "noisy pneumothorax."<sup>12</sup> For practical purposes the chest radiograms were not performed in expiration, when a small pneumothorax would more often be evident.

3. Pleural effusion was seen in 15 cases, in 12 cases on the left side only, in one on the right side, and in two bilateral. It always appeared to be minimal, although no radiographs were taken with the patient in the lateral recumbent position.

4. Pulmonary complications in the form of small basal, atelectatic streaks were seen on the left side in six cases, and on both sides in two cases. Only one case had fever exceeding 38 C. Two cases showed clinical and radiologic signs of bronchopneumonia 1 or 2 days after the puncture.

In cases with effusion and parenchymatous changes there were no abnormalities in the radiographs immediately after puncture. In contrast, pneumothorax was detectable as often in these as in the radiograms a day later. This observation may explain the lung changes: the immobilization for 12 to 14 hours after puncture plus slight chest pain may cause a superficial ventilation. The observed changes soon disappeared.

5. Late disturbances of heart rhythm. Atrial fibrillation began one and two days after the puncture in two cases with combined mitral and aortic valvular disease. There may have been no relation between the puncture and the fibrillation, although late onset of arrhythmia has been noted often after noncardiac thoracic surgery.<sup>13</sup>

#### Major Complications

This group includes ventricular fibrillation, cardiac tamponade, faulty contrast injection, and cerebral complications (table 4). Some of our cases were briefly reported earlier.<sup>11</sup>

1. Ventricular fibrillation occurred once, in a 32-year-old woman with aortic stenosis, aortic insufficiency, and slight aortic coarctation. It was difficult to reach the ventricular cavity and at the second puncture ventricular fibrillation appeared. Thoracotomy was immediately performed, and after cardiac massage and defibrillation sinus rhythm was rapidly restored. Left ventricular puncture was then made on the exposed heart, but no contrast medium was injected. The patient recovered.

2. Cardiac tamponade occurred in six cases, twice with fatal outcome. The first case was a 55-year-old man with severe calcific aortic stenosis. For technical reasons no contrast medium could be injected. When returned to the ward he developed signs of cardiac tamponade, and thoracotomy was performed when he developed cardiac standstill. In spite of cardiac massage and acute surgical dilatation of the aortic valve, spontaneous cardiac activity was not restored. He had a large hemopericardium and autopsy revealed gross hypertrophy of the left ventricle except at a small region near the apex. Here the wall was only a few millimeters thick, and this was unfortunately the puncture site. Microscopically this area showed interstitial fibrosis.

The second fatal case was a 47-year-old woman with severe mitral insufficiency. She was severely ill with pulmonary hypertension, a heart size of 1,760 ml./M.<sup>2</sup> body surface area, and a very low capacity for work. About 2 hours after the puncture with left ventricular angiography, she became hypotensive, and soon afterwards the heart stopped. On thoracotomy cardiac tamponade was found but cardiac massage was without success.

In a third case, a 27-year-old man with a small ventricular septal defect, the puncture was successful but no contrast medium was injected because the tubing ruptured. Beginning from the second day he was febrile. Repeated chest radiographs showed at first only

Table 4

*Major Complications in 142 Left Ventricular Punctures*

	Number	
	Fatal	Nonfatal
Ventricular fibrillation		1
Cardiac tamponade	2	4
Faulty contrast injection	1	3
Cerebral complications	2	2

left pleural effusion; on the sixth day, enlargement of the cardiac silhouette. An electrocardiogram taken this day showed frequent ventricular extrasystoles and S-T elevations indicative of pericarditis. On pericardial puncture on the seventh day only 70 ml. of blood were obtained. On the tenth day he fainted in bed. The area of absolute cardiac dullness was increased and another pericardial puncture on the eleventh day yielded 120 ml. of blood, but the patient became unconscious and thoracotomy was performed. The pericardium was found to contain 1,400 ml. of blood. There was right ventricular hypertrophy with clockwise rotation of the heart. Consequently the descending branch of the left coronary artery lay near the apex and had been injured by the needle. The patient made a good recovery but 3 months later he was hospitalized once more because of chest pain and fever. The heart size had increased and a thoracotomy was performed again, since puncture failed. Besides a pleural exudate a framework was found inside the pericardial cavity, containing blood-stained fluid. To prevent constricting pericarditis a pericardectomy was deemed necessary. He finally recovered after a second relapse with fever and signs of myocarditis. We interpreted this case as cardiac tamponade which was atypical, due to the slow oozing of blood from the injured vessel, complicated with a perimyocarditis with doubtful relation to the puncture.

In a fourth case 100 ml. of blood were evacuated by pericardiocentesis a few minutes after the procedure and resulted in improvement. In two other cases only 50 ml. of blood could be evacuated. In at least one of these there were obvious clinical signs of cardiac



Figure 1

*Left ventricular angiogram, frontal projection. The contrast medium is mainly deposited in the myocardium and in the pericardial sac. Early contrast filling of the coronary veins. No contrast medium in the aorta.*

tamponade that promptly subsided afterwards.

3. Faulty contrast injection caused the death of a 9-month-old boy with atypical tetralogy of Fallot. On suspicion of aortic stenosis the puncture was performed and at the third attempt the needle entered the left ventricle. In spite of a previous free pressure curve, the electrocardiogram showed deep S-deflections and S-T depressions during the contrast injection. The angiogram and a chest radiograph 1½ hours later showed contrast medium in the left myocardium (fig. 1). He did well the first hour but then rapidly deteriorated. Because of cardiac standstill thoracotomy was performed but no contractions of the left ventricle could be evoked. The pathologic examination showed hemorrhage in the posterior wall of the left ventricle, with 3-cm. rupture of the endocardium.

In two cases the angiogram showed that small amounts of contrast medium had been injected into the myocardium. However, no contrast medium was detectable on the radiographs taken some minutes later, and there were no clinical symptoms. In a fourth case a small amount of contrast medium was injected into the pericardial sac. There were no sequelae.

Table 5  
The Reported Occurrence of Major Complications in Left Ventricular Puncture

Authors	Number Angio- gram	Number No angio- gram	Ventric- ular fibril- lation	Cardiac tamponade	Faulty (= extravascular) contrast injection	Needle diam. mm.
<b>I. Subxiphoid method</b>						
Nunez et al., 1951 <sup>15</sup>	45					
Ponsdomenech et al., 1951 <sup>16</sup>	56				1	1.7
Smith et al., 1954 <sup>17</sup>	6		1			1.5
Clegg et al., 1955 <sup>18</sup>	8		1			
Smith et al., 1956 <sup>19</sup>	31		1			
McCaughan et al., 1957 <sup>20</sup>	31				2	
Lehman et al., 1957 <sup>21</sup>	69				8	1.5
Lehman et al., 1959 <sup>22</sup>	230				5 (2)*	
Lehman, 1959 <sup>23</sup>	>300			3	3 (3)	
Greenberg et al., 1960 <sup>24</sup>	41				6	
	Total	459		1	3	17 (3)
<b>II. Intercostal method</b>						
Nuvoli, 1936 <sup>25</sup>		1				
Buchbinder et al., 1949 <sup>26</sup>		1				
Brock et al., 1956 <sup>10</sup>		24				1.25
Fleming et al., 1957 <sup>27</sup>		28				
Fleming et al., 1958 <sup>28</sup>		115		3 (2)		1.5
Ross, 1959 <sup>29</sup>		184		2 (2)		
Morrow et al., 1958 <sup>20</sup>		7		1		
Yu et al., 1958 <sup>31</sup>		30				1.1
Greene et al., 1958 <sup>22</sup>		23				0.8
Conolly, 1959 <sup>22</sup>		>50				0.9—1.1
Botham et al., 1959 <sup>24</sup>		1		1		1.25
Björk et al., 1961	133	9	1	6 (2)	4 (1)	1.55
	Total	133	305	1	11 (4)	4 (1)

\*The figure in parentheses designate fatal outcome.

In two cases, a 36-year-old woman and a 55-year-old man, both with combined mitral-aortic valvular disease, there was a disorientation for some hours afterwards with amnesia. There were no other neurologic signs and they recovered completely. The probable genesis was a reaction to the contrast medium. In the first case, however, there were some signs of cardiac tamponade, too; and pericardial puncture yielded 50 ml. of blood.

#### Discussion

The reported complications of left ventricular puncture in the literature are listed in table 5. They are divided according to the method of puncture—subxiphoid or intercostal. As is evident from the table, practically all workers with the subxiphoid method have performed left ventricular angiography

whereas our group has employed the intercostal method. The different consecutive reports by one and the same team are listed together, as some of the major but nonfatal complications are not repeated in their latest reports.

*Ventricular fibrillation* that necessitated thoracotomy occurred twice, both with recovery after cardiac massage. In the case of Smith et al.<sup>19</sup> contrast medium was injected slowly and near the origin of the left coronary artery. In our case no contrast medium was injected but repeated punctures were made. The risk of ventricular fibrillation emphasizes the absolute necessity of a continuous electrocardiogram during the procedure. Impending ventricular fibrillation is sometimes heralded by multiple ventricular extrasystoles; if it is established, cardiac resuscitation must be per-

formed without delay. As prophylaxis 100 per cent oxygen breathing and the administration of quinidine or procaine amide might be of value. Premedication with drugs containing atropine may cause tachycardia and ventricular extrasystoles.

Fatal cardiac tamponade seems to occur with either method. Lehman reports three successfully treated cases among more than 300 left ventricular punctures.<sup>23</sup> In a series of 184 intercostal punctures Ross<sup>29</sup> reports two fatal cases, a 61-year-old woman with arteriosclerotic heart disease without aortic stenosis, and a 57-year-old man with aortic stenosis. In both cases the procedure was somewhat protracted due to attempted aortic catheterization. In the first case the needle entered the left ventricle through a 3-mm. thick fibrous part of the myocardium as in our fatal case. In a third case<sup>28</sup> signs of cardiac tamponade appeared suddenly on the fourth day after the puncture but then spontaneously subsided. Morrow et al.<sup>30</sup> observed one case of cardiac tamponade in seven direct left ventricular punctures. Botham et al.<sup>34</sup> reported one case of cardiac tamponade that necessitated thoracotomy. The needle had been left in situ for 15 minutes.

In our two fatal cases cardiac tamponade was at once suspected but unfortunately thoracotomy (or pericardial puncture) was not performed until cardiac arrest suddenly appeared. It is possible that immediate intervention might have prevented the fatal outcome.

It seems probable that the amount of blood in the pericardial cavity can be rather large without causing signs of tamponade. Of the 69 cases reported by Lehman et al.<sup>21</sup> 35 were operated upon 1 to 20 days after the puncture. Eleven cases were found to have 50 to 100 ml. of blood in the pericardium, two cases 100 to 200 ml., and in one case 300 ml. In no case were there clinical signs of cardiac tamponade. Green et al.<sup>32</sup> reported thoracotomy in 18 cases immediately after the puncture and generally found 75 to 100 ml. of blood; in one case, 150 ml. None of these cases showed clinical signs of cardiac tamponade in spite of a

rather large hemopericardium. The explanation is probably that the bleeding had been slow. On the other hand, small but rapid bleeding might cause signs of tamponade.

In the available literature no report has been seen of a lacerated coronary vessel as the cause of hemopericardium after left ventricular puncture. This was the mechanism in one of our cases (L.B.) in which thoracotomy was performed. Preoperatively there had been a strong suspicion of a ventricular septal defect.

Hemopericardium with cardiac tamponade should be suspected when a fall in blood pressure reappears some minutes after the procedure. In our experience the electrocardiogram has not been very helpful. Only standard lead II has been used and there have been no diagnostic ST-T alterations. Likewise the chest radiographs taken about 10 minutes after the puncture have been normal in all cases, with unaltered size silhouette. Thus, it is not probable that studying the heart size during the Valsalva maneuver would be helpful for the diagnosis of pericardial effusion. We have not been observant regarding the presence of the pulsus paradoxus, as is described in pericardial effusion.

When cardiac tamponade is suspected, urgent therapy is necessary. Pericardiocentesis should be carried out as rapidly as possible, in the ward or in the cardiac laboratory. If immediate, significant, and lasting rise in blood pressure is not obtained, another puncture is performed. If the patient still is hypotensive, a thoracotomy is performed at once. Bishop et al.<sup>35</sup> have used the electrocardiogram as a safeguard during pericardial puncture. An argument in favor of thoracotomy might be the possibility of finding and properly treating the bleeding site.

The one case of *faulty (extravascular) contrast injection* of Ponsdomenech et al.<sup>16</sup> had the contrast deposited in the pericardium with no untoward effects. The case of Smith et al.<sup>19</sup> with ventricular fibrillation, cited above, was due to contrast injection near a coronary artery. The same authors reported that the needle once entered a distended stomach, appar-

ently with no ill effects.<sup>17</sup> Lehman<sup>23</sup> lost three cases due to intramyocardial injection, one case not until 6 months later and due to bolus of contrast substance in the septum causing heart block. Seven other cases survived. Greenberg et al.<sup>24</sup> reported six faulty injections, four into the myocardium, one into the pericardium, and one into both. There were transient electrocardiographic abnormalities but no sequelae. Our fatal case was a 9-month-old boy; in infants this procedure should be avoided. In a 6-month-old girl with mitral insufficiency and ventricular septal defect, however, the puncture with angiography was successful but the contrast medium was injected manually. In no case did we erroneously inject into the right ventricle. This occurred in 12 injections by Lehman et al.<sup>22</sup>

Certain precautions should be undertaken to prevent this complication.<sup>17, 21</sup> The needle must be short-beveled, preferably with a blunt end and a sharp mandrin. Side-holes might give better contrast mixing but would be more dangerous because a free pressure curve would not guarantee a completely free position in the cavity. The pressure curve should be free immediately before the injection (as it was, however, in our fatal case). The patient must hold his breath during the 2 to 3 seconds of injection. In agitated patients general anesthesia might be preferred; in infants, it is necessary. The pressure curve must be unchanged and free when the needle is moved in different directions. Lehman et al.<sup>21</sup> do a gradual withdrawal until the curve becomes damped, then push the needle in about 0.5 cm. A test injection of 1 ml. of contrast solution under electrocardiographic control is used by McCaughan et al.<sup>20</sup> as a safeguard against extravascular injection. These precautionary measures should be taken immediately before angiography, and in the same phase of respiration used during the contrast injection.

The whole procedure should not take longer than about 5 minutes. The gage of the needle seems to be of some importance, as is seen from table 5. Fleming et al.<sup>25</sup> experienced three cases with cardiac tamponade when

changing from a needle with a 1.25-mm. outer diameter, to one with 1.5 mm., in order to facilitate catheterization of the aorta. In these cases, however, the prolonged time may be the chief cause. On the other hand, a needle with smaller diameter is less suitable for angiography, as the contrast medium will be injected too slowly.

#### Summary and Conclusions

In a series of 142 percutaneous left ventricular punctures we have encountered 11 cases of major complications with three fatalities. About 900 left ventricular punctures are reviewed, with a total of 8 fatal complications, i.e., less than 1 per cent. The fatal cases were to the same extent due equally to cardiac tamponade and intramyocardial deposition of contrast material.

To prevent cardiac tamponade the needle should be as small as practicable, and the needle left in the heart as short a time as possible. Great care should be exercised when ischemic heart disease is suspected. Cardiac tamponade requires prompt diagnosis and immediate treatment.

The risk of faulty contrast deposition may be minimized by ensuring the free position of the needle end in the ventricular cavity immediately before angiography. This risk is larger in small infants with a small ventricle.

The puncture always must be performed with continuous electrocardiographic control. Ventricular fibrillation must be immediately diagnosed and treated, but fortunately it is rare.

The percutaneous puncture can be performed either by the subxiphoid or the intercostal method. The frequency of cardiac tamponade was nearly four times greater with the latter method. On the other hand there is a higher frequency of extravasal contrast injection with the subxiphoid method. The question of which method is safest cannot be answered yet.

In our opinion, percutaneous left ventricular puncture combined with selective left ventricular angiography is a practical method that gives valuable information re-

garding rheumatic heart disease, especially aortic stenosis and mitral insufficiency. It is, however, associated with a definite morbidity, some of which cannot be avoided. This method should therefore be performed only in the investigation of cases for major heart surgery. At least in cases without tight aortic stenosis retrograde aortic catheterization of the left ventricle is, according to our experience in the last year, a more secure and equally informative method. Retrograde left ventricular angiography may immediately be followed by aortography to evaluate competence of the aortic valve.

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### The Early History of Precision in Medicine

The latter part of the 16th and the first half of the 17th centuries was a germinal period in medicine. It saw advances in anatomy and physiology, which led up to Harvey's splendid discovery. It saw, too, the failure of his thesis to influence medical practice immediately or largely. The same period in Italy beheld the first attempts at precision as regards temperature and the study of the pulse. This was the birth era of instrumental accuracy in medicine, but many a day went by before the infant attained to useful manhood. Most strange it is that the seeds of scientific thought as to the first heat records and the pendulum were cultivated in the garden of medicine. Between 1593 and 1597 Galileo, sometime a student of medicine, invented the crude open thermometer or thermoscope....

The thermometer of Galileo was, as I have drawn it (fig. 2), a tube of glass, open below and ending above in a bulb. This bulb having been warmed the open end of the tube was set in water, so that as the bulb cooled, the water rose in the tube. Then any heat applied to the bulb caused the water to descend, the reverse of that which occurs in the more modern instrument. This coarse thermoscope was obviously a barometer as well as a rude measurer of the change of temperature. A slight change in the weight of the atmosphere might easily neutralize an increase of heat. It was not an accurate instrument, nor does Galileo seem to have rated it highly since he nowhere mentions it in his works. Others thought more of it. The approximate date of this invention is set for us by one Padre Benedetto Castelli, in a letter about the treatment of a wounded man, written to one Cesarini, in 1638. He calls to mind the fact that Galileo had thirty-five years before shown him the air thermometer.—S. WEIR MITCHELL, M.D. The Early History of Instrumental Precision in Medicine, *Transactions of the Congress of American Physicians and Surgeons*, Second Triennial Session held at Washington, D.C., 1891. New Haven, The Congress, 1892, p. 166.



## Study of the Mechanism of Hyperlipemia

### Serum Chylomicron Fatty Acid Patterns of Hyperlipemic Patients before and after the Ingestion of Different Food Fats

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CURRENT epidemiologic, experimental and clinicopathologic studies indicate that elevation of serum lipid levels caused by hereditary, nutritional, or pathologic factors are correlated with an increased frequency of atherosclerosis. Among the various abnormal plasma lipid patterns described in patients with coronary artery disease, an increase in the fasting triglyceride-rich chylomicrons (hyperlipemia)<sup>1,2</sup> together with a prolonged and intensified alimentary lipemia<sup>3-6</sup> appears to constitute the best laboratory index of probability of the disease. In examining this correlation from another direction, many investigators have reported an increased incidence of coronary atherosclerosis among patients with idiopathic hyperlipemia and among their family members.<sup>7-11</sup>

In the present study, an attempt has been made to identify the source of the plasma chylomicrons in hyperlipemic patients who are known to have coronary or peripheral arterial diseases by analyzing the compositions of their serum chylomicron fatty acids during fasting and at various intervals following the feeding of cream, corn, and coconut oils.

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#### Experimental Procedure and Methods

A series of ten patients, eight men and two women, with various degrees of hyperlipemia and clinical evidences of coronary or peripheral arterial disease was used in this study. The ages of these patients ranged between 36 and 61 years. While on a full American diet, their fasting serum triglyceride concentrations were in the range of 336 to 3,020 mg. per cent.

Initially, all patients were placed on a full diet for 4 to 6 weeks. After this preparatory period, three fasting blood samples (obtained 12 hours after the previous meal) were taken at 3-day intervals from each patient for determinations of plasma lipid concentrations and for isolation of serum chylomicrons. During the ensuing 10 to 14 days, each patient was fed three special breakfasts, one of 40 per cent cream, one of coconut oil, and one of corn oil emulsion. The dose was 1.5 Gm. of cream fat or oil per Kg. of body weight. A second cream feeding of 2.5 Gm. per Kg. of body weight was given to three of the patients after the completion of this series of three tests. In each test, blood samples were taken during fasting and at 2- to 3-hour intervals for 12 hours after ingestion of the fatty meal.

After completing the feeding experiments, eight patients were placed on a low-fat diet estimated to contain about 1,600 to 1,800 calories and less than 35 Gm. of total fat per day for 8 to 24 weeks. Fish, shell-fish, chicken, and turkey were the chief sources of dietary proteins and fats during this period on a low-fat diet. The remaining two patients were given a diet consisting only of rice, fruit, and vegetables, 1,000 to 1,200 calories per day, for 4 to 8 weeks. After about 3 weeks of the dietary program, fasting blood samples were taken from each patient at weekly intervals for triglyceride estimations. Analyses of the serum chylomicron fatty acid content were performed after their fasting serum triglyceride concentrations had been decreased to or below 300 mg. per cent.

Six male medical students and four female laboratory technicians from 18 to 26 years of age were studied as controls. All subjects were clinically healthy and consumed a full American diet.

**Table 1**  
*Fatty Acid Composition of Fasting Serum Chylomicrons and of Depot Fat in Ten Young and Healthy Subjects (Mean and Range in Per Cent)\**

Fatty acids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2
<i>Source</i>								
Serum chylomicra, fasting (full diet)								
Cream layer	1.4 (1.0-2.9)	2.6 (1.5-3.7)	1.5 (0.7-2.3)	31.1 (29.8-36.2)	3.8 (2.2-5.4)	9.2 (6.1-12.8)	27.6 (24.2-31.3)	22.7 (17.8-25.0)
Saline layer	1.3 (0.7-2.3)	2.5 (1.5-3.1)	1.0 (0.7-2.1)	34.9 (30.8-37.6)	3.7 (2.8-6.6)	11.2 (6.3-13.1)	22.4 (19.8-27.8)	23.0 (18.0-28.7)
Depot fat	2.0 (1.1-3.9)	5.6 (4.4-8.0)	2.2 (1.7-3.6)	24.7 (22.7-30.6)	5.8 (5.5-6.1)	4.6 (3.7-6.5)	44.9 (41.9-49.4)	10.1 (8.4-11.9)

\*In serum, the very light "cream" and the heavier "saline" layers are analyzed separately.

**Table 2**  
*Clinical Data and Serum Lipid Levels of Ten Hyperlipemic Patients*

Patient	Sex	Age	Coronary artery disease	Peripheral vascular disease	Blood lipid ranges (mg. per cent)		
					Total cholesterol	Phospholipid	Triglyceride
H.A.	M	61	—	+	206-262	180-238	336-473
R.M.	M	52	+	—	222-276	204-301	410-580
H.N.	F	52	+	—	535-614	583-643	1,780-2,230
D.H.	F	41	+	+	430-494	298-343	393-481
J.H.	M	38	+	+	472-628	583-742	984-1,879
H.S.	M	50	—	+	382-394	333-395	520-725
M.C.	M	61	+	+	219-280	385-398	1,250-1,658
C.W.	M	48	+	+	317-369	355-370	376-556
M.G.	M	54	+	—	442-464	410-455	2,718-3,020
G.H.	M	36	+	—	304-366	373-410	405-590

In this group, the special breakfast program was instituted as described in the hyperlipemic group, after an initial sample of blood had been taken in a manner similar to that used in the hyperlipemic patients. They were bled at 2- to 3-hour intervals for 6 hours following ingestion of each fatty meal. Blood samples were also taken from two patients and three normal subjects at the peak of their alimentary lipemias, following ingestion of a full-course dinner, for determination of their serum chylomicron fatty acid patterns.

Subcutaneous fat was obtained in each subject by a small biopsy. The tissue obtained was first washed free of blood by vigorous shaking in several portions of saline. It was then ground into a fine suspension and centrifuged.

Triglycerides and chylomicrons were isolated from the tissue emulsion and serum samples by flotation into the previously layered saline solution, density 1.006, at 58,000 x g. for 30 minutes. Two gross fractions of saline floatable lipids were collected from each serum specimen: (a) a "cream" layer at the top of the tube and (b) a relatively clear or slightly turbid "saline" layer. The "cream" layer was collected by gentle suction and purified by a further washing in saline. Because of the

known difficulty in effecting a clear-cut separation between chylomicrons and very low density lipoproteins, both the purified cream layer (containing mostly chylomicrons and little very low density lipoproteins) and its subnatant saline phase (containing very low density lipoproteins and variable proportions of chylomicrons) of each specimen of serum were subjected to chromatographic analysis separately. The two fractions will be identified as "cream" and "saline" layers respectively henceforth.

Samples of cream, corn oil, coconut oil, serum chylomicron fractions, and tissue fat were extracted with chloroform/methanol according to the method described by Bragdon.<sup>12</sup> Methyl esters of the fatty acid mixtures were prepared by the method of Stoffel and his associates.<sup>13</sup> The methyl esters formed were extracted into redistilled hexane and then concentrated by evaporation under nitrogen. The fatty acid composition of each sample was analyzed by gas-liquid chromatography.

Gas chromatography was performed according to the procedure outlined by Farquhar and his associates<sup>14</sup> with use of a Barber Coleman Model 10 chromatograph with an argon-ionization detector.<sup>15</sup> The standard 6-foot glass column was

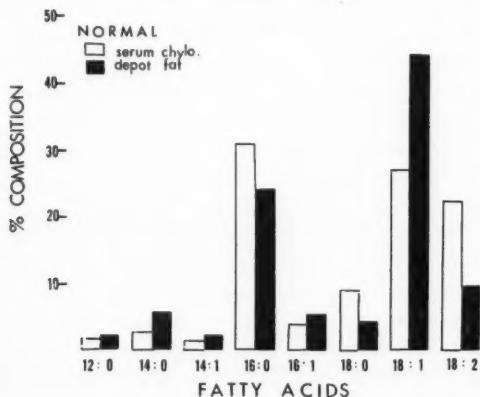


Figure 1

Hollow bars present the mean percentages of major fatty acids found in the fasting serum chylomicrons of 10 healthy young controls. The percentage composition of fatty acids found in their depot fat is shown in solid bars.

packed with chromosorb W that had been coated with 15 per cent ethylene glycol succinate as the stationary phase. Analysis was carried out at 186 C., with argon flowing at 100 ml. per minute and the voltage of the amplifier was set at 500 volts. The area of each of the major fatty acid peaks from C<sub>12</sub> to C<sub>18</sub> in the chromatogram was calculated by multiplying the height by half of the base.<sup>16</sup> The areas were then totaled and the percentage of each component was determined. No attempt was made to measure or identify the minor constituents.

In this report, the fatty acids are identified by a system of dual symbols to indicate the chain length and number of double bonds, as suggested by Dole and his associates.<sup>17</sup> The degree of lipemia was estimated by measuring the triglyceride concentration of the serum by the method of Van Handel and Zilversmit.<sup>18</sup>

### Results

#### Fasting State

The fasting serum of normal subjects contains few chylomicrons. A satisfactory analysis can usually be made, however, on a 20-ml. sample. The mean and the range of fatty acid composition found in the fasting serum chylomicrons of the 10 control subjects while they were eating a full diet are presented in table 1. Four fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), constitute over 90 per cent of the total major fatty acid components found in each of the

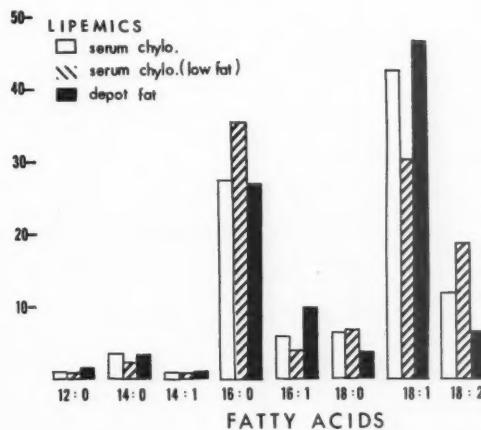


Figure 2

Hollow bars represent the mean percentages of the major fatty acids found in the fasting serum chylomicrons of 10 hyperlipemic patients. The mean percentage compositions of these fatty acids in the serum following a period of low-fat diet are shown in hatched bars; those of their depot fat, in solid bars.

saline floatable fractions ("cream" and "saline" layers) of the serum. Although minor differences in mean fatty acid composition were observed in the two saline floatable serum lipid fractions, generally speaking their fatty acid patterns were fairly similar. This is most likely due to the fact that the fasting serum of normal subjects contains few very light chylomicrons of dietary origin. On the other hand, the serum chylomicron fatty acid composition of these normal subjects is quite different from that of their depot fat, which shows a relatively large increase in 18:1 and relative decreases in 16:0, 18:0, and 18:2 acids. A comparison of the chemical compositions of the serum chylomicrons and that of the depot fat of the normal controls is graphically presented in figure 1.

The clinical diagnoses and blood lipid concentration data of the 10 hyperlipemic patients in this series, while consuming a full diet, are summarized in table 2. All 10 patients had clinical evidence of coronary or peripheral arterial disease.

The fasting serum chylomicron fatty acid

Table 3  
*Fatty Acid Composition of Serum Chylomicron and Depot Fat in Hyperlipemic Patients  
 after Fat Meal (Per Cent Fatty Acids in Means and Ranges)*

Fatty acids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2
<i>Source</i>								
Serum chylomicra, fasting (full diet)								
Cream layer	0.9 (0.3-1.2)	3.5 (2.7-4.2)	0.9 (0.3-1.4)	27.6 (25.3-29.1)	5.9 (2.9-6.9)	6.4 (5.9-8.0)	42.9 (41.4-45.1)	11.9 (8.4-15.3)
Saline layer	0.8 (0.5-1.3)	2.0 (1.4-2.4)	0.6 (0.0-0.7)	37.4 (34.3-39.7)	3.7 (2.1-6.0)	7.4 (6.1-7.5)	31.8 (28.3-35.3)	16.3 (15.4-19.4)
Serum chylomicra, absorptive (full diet)								
Cream layer	2.1 (2.0-2.2)	4.8 (4.2-5.4)	1.0 (0.9-1.1)	26.2 (25.3-27.0)	2.3 (1.8-2.8)	8.4 (8.3-8.6)	45.6 (44.0-47.1)	9.6 (8.5-10.8)
Serum chylomicra, fasting (low fat diet)								
Cream layer	0.9 (0.4-1.7)	2.2 (1.3-3.3)	0.7 (0.1-1.4)	35.8 (31.5-39.1)	4.0 (2.7-5.2)	7.0 (4.4-10.4)	30.4 (28.9-33.7)	19.0 (14.2-23.3)
Saline layer	0.9 (0.4-2.1)	2.3 (1.1-3.3)	0.8 (0.5-1.4)	36.9 (33.0-42.0)	3.7 (2.2-3.9)	8.7 (6.4-15.4)	26.5 (19.6-33.2)	20.2 (13.3-25.4)
Serum chylomicra, fasting (rice diet)								
Cream layer	0.4 (0.3-0.5)	1.6 (1.4-1.9)	0.3 (0.2-0.4)	31.3 (29.6-33.1)	7.5 (6.0-9.0)	3.8 (3.2-4.3)	46.1 (45.3-47.0)	9.0 (8.6-9.3)
Saline layer	0.7 (0.6-0.8)	2.1 (2.0-2.2)	0.4 (0.3-0.5)	32.2 (32.0-32.3)	9.0 (7.9-10.0)	4.5 (4.0-4.9)	41.6 (41.5-41.8)	9.5 (8.3-10.7)
Depot fat	1.5 (0.9-1.9)	3.3 (2.2-5.8)	1.0 (0.7-1.8)	27.2 (20.8-31.1)	9.9 (8.3-14.3)	3.5 (2.1-5.6)	46.9 (43.7-49.5)	6.7 (4.5-10.1)

pattern of these patients was found to vary with the dietary program they had received. The means and the ranges of the serum chylomicron fatty acid components of these patients while consuming a full American type of diet for 4 or more weeks (10 patients), a low-fat diet for 3 to 6 weeks (eight patients), and a rice, fruit, and vegetable diet for 4 to 8 weeks (two patients) are listed in table 3. Analyses made on the depot fat and on serum chylomicrons obtained at the height of absorption of a regular meal are also included, for comparative study. While on a full diet, the fasting sera of the hyperlipemics differed from those of the normal controls in these respects: (a) following ultracentrifugation, a thick layer of butter-like substance was floated on top of the saline phase; (b) the fatty acid pattern of this cream layer showed a relative increase in 18:1 and relative decreases in 16:0, 18:0, and 18:2 fractions; (c) this chylomicron

fatty acid pattern resembled that of the depot fat and also that of alimentary lipemia following the ingestion of a regular meal, either by a normal or a hyperlipemic subject; (d) considerable differences in the fatty acid composition between the lipid materials in the "cream" and "saline" layers were observed.

At various intervals, following the institution of a low-fat dietary regimen, the fatty acid pattern of the fasting serum chylomicrons of these patients showed progressive changes toward that of the normal controls. The composition of the depot fat, however, was unchanged. The fatty acid patterns of the very light serum chylomicrons observed in these patients before and after the use of low-fat diet and the pattern obtained from the depot fat are compared in the bar graphs in figure 2.

While they were receiving a rice, fruit, and vegetable diet with a total intake of 1,000

to 1,200 calories per day, the fasting serum fatty acid pattern of two hyperlipemic subjects became practically identical with that of their depot fat, suggestive of mobilization of glycerides from the depot. In contrast to dietary fat, which tended to be concentrated in the "cream" layer, the mobilized liquid appeared to distribute itself fairly evenly in both the "cream" and the "saline" layers of the serum chylomicrons. Hence, in this condition, the fatty acid patterns of the two serum chylomicron fractions and the depot fat were fairly similar. Essentially the same findings were obtained in three patients with severe malabsorption syndrome. These changes were not observed in two hypercholesterolemic patients who went on a rice and fruit diet for 6 weeks, and in two other hyperlipemic patients who were kept on a rice diet for more than 6 months, but were maintained on a total intake of more than 2,000 calories per day.

#### Feeding Experiments

Analyses of the serum chylomicrons of normal subjects receiving the test meal fat and obtained at the peak of alimentary lipemia, which occurs in most cases 3 hours, and at times 5 hours, after the ingestion of a fatty meal, are shown in table 4. The data emphasize the role of dietary fat in the production of alimentary lipemia (fig. 3). When 40 per cent cream was fed, the fatty acid composition of the serum chylomicrons was changed to resemble that of milk fat. When corn oil,\* which contains mainly longer-chain fatty acids, and coconut oil, which contains a larger amount of short-chain acids, were fed, striking changes oc-

\*Kindly supplied by the Corn Products Refining Company, Argo, Illinois.

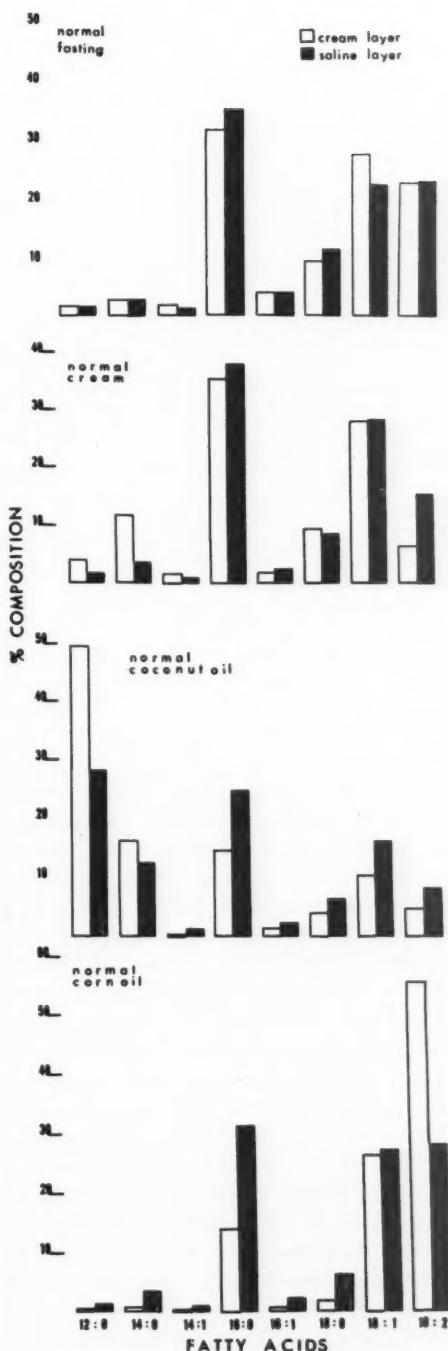


Figure 3

*Changes in the mean percentage fatty acid composition of very light serum chylomicrons (hollow bars) and the saline floatable heavier density particles (solid bars) before and after cream, coconut oil, and corn oil feedings in 10 normal controls.*

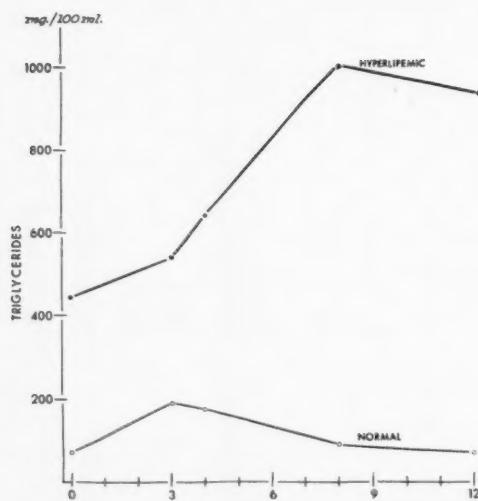


Figure 4

Mean serum triglyceride curves of the control and hyperlipemic groups following fatty meal ingestions are compared.

curred in the serum chylomicron composition in the direction of the administered oils. Cream, corn oil, and coconut oil feedings given to hyperlipemic patients resulted in an intensified and prolonged alimentary lipemia. Their elevated serum triglyceride concentration curve continued to rise at a time interval when the much lower curve of the controls was dipping toward its fasting level (fig. 4). This phenomenon was exaggerated by increasing the dosage of the administered fat from 1.5 to 2.5 Gm. per Kg. of body weight. The data on serum chylomicron fatty acid composition during peak intestinal absorption, 6 to 12 hours following cream, corn oil, and coconut oil feedings, are shown in table 4 and figure 5. Like the normal controls, the fatty acid patterns of the very light serum chylomicrons of these patients were also practically identical to those of the administered fat. This remarkable degree of similarity between the compositions of serum chylomicrons and of food fats was achieved in these patients, despite the presence of severe degrees of hyperlipemia.

### Discussion

Isolation of chylomicrons from serum is usually made by layering the latter under physiologic saline and centrifuging it for 10 to 30 minutes at 9,000 to 100,000 g.<sup>19, 20</sup> The centrifugal force concentrates the very lowest density particles to form a "cream" layer at the top of the tube, and floats the heavier molecules into the saline phase. In view of the known inhomogeneity of chylomicrons and the inability to obtain a clean separation between them and the very low density lipoproteins,<sup>21, 22</sup> analyses of the fatty acid composition were carried out in both the "cream" and the "saline" layers of each blood sample in the present study.

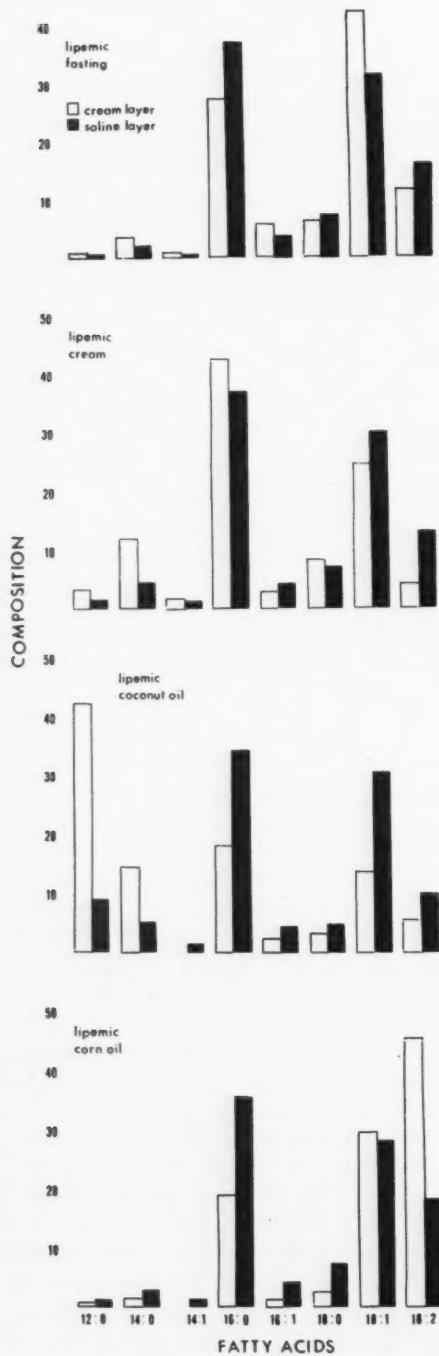
In their study of the fatty acid pattern of plasma chylomicrons of normal subjects during alimentary lipemia, Dole and his associates<sup>17</sup> have stressed the relative stability of the serum chylomicron fatty acid composition during the period of fat absorption. They suggested that dietary fat did not contribute significantly to alimentary lipemia. In similar feeding experiments made on rats and normal human subjects, however, Bragdon and Karmen<sup>23</sup> found a close similarity in fatty acid composition between the serum chylomicrons and the ingested fats. The data obtained in this present study are confirmatory of the work of the latter group in that, following fat feeding, the serum chylomicron fatty acid pattern of normal subjects was found to be practically identical with that of those fed food fat. This observation was extended to the group of hyperlipemic patients. We are also in agreement with Bragdon and Karmen that unless suitable precautions are taken, the collected creamy layer of chylomicrons could be heavily contaminated with lipid particles not of immediate dietary origin. In fact, washing and resuspending the carefully aspirated "cream" layer in larger amounts of physiologic saline has been found to be more important than either the duration or the force of centrifugation used in the isolation of a relatively pure light chylomicron fraction.

**Table 4**  
*Fatty Acid Composition of Serum Chylomicron and Depot Fat in Hyperlipemic Patients after Ingestion of Cream and Coconut and Corn Oils*

Fatty acids	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2
<i>Source</i>								
1. Dairy cream	4.7	17.3	2.2	40.5	1.8	9.5	23.1	0.8
Serum chylomicra								
(a) controls								
Cream layer	4.1 (2.1-6.3)	11.9 (9.2-14.4)	1.7 (1.5-1.9)	35.9 (31.6-42.1)	1.0 (1.7-2.3)	9.6 (8.1-12.0)	28.2 (24.7-32.5)	6.6 (4.3-11.0)
Saline layer	1.7 (1.1-2.0)	3.7 (2.6-4.2)	0.9 (0.4-1.6)	38.5 (33.0-41.7)	2.5 (1.6-3.7)	8.7 (4.8-11.8)	28.6 (24.4-34.0)	15.4 (10.5-19.4)
Serum chylomicra								
(b) hyperlipemics								
Cream layer	3.2 (2.0-4.0)	11.9 (10.0-14.5)	1.5 (0.9-2.0)	42.8 (39.4-45.4)	2.9 (2.0-4.5)	8.5 (7.9-10.0)	24.8 (23.0-29.1)	4.3 (3.0-5.7)
Saline layer	1.8 (0.9-3.3)	4.6 (2.7-8.9)	1.3 (0.8-4.6)	37.3 (33.9-43.4)	4.3 (2.9-5.8)	7.3 (5.0-11.2)	30.4 (25.7-38.1)	13.2 (10.0-20.8)
2. Coconut Oil	72.5	16.2	—	5.6	—	1.6	2.8	1.2
Serum chylomicra								
(a) controls								
Cream layer	49.7 (38.2-65.8)	16.1 (10.9-20.7)	—	14.6 (12.3-20.8)	1.1 (0.7-1.6)	4.0 (2.4-6.2)	10.0 (8.8-15.6)	4.6 (2.4-6.6)
Saline layer	28.1 (27.4-28.8)	12.5 (11.1-14.0)	1.3 (0.5-2.5)	24.8 (23.1-26.6)	2.2 (1.1-3.1)	6.5 (3.8-10.3)	16.2 (13.9-20.1)	8.4 (4.7-11.6)
Serum chylomicra								
(b) hyperlipemics								
Cream layer	42.2 (32.7-59.2)	14.4 (11.3-20.7)	—	18.2 (13.4-20.7)	2.3 (1.1-5.3)	3.2 (2.0-4.6)	13.9 (7.9-20.9)	5.7 (3.2-8.6)
Saline layer	9.0 (5.7-11.6)	5.1 (4.0-6.3)	1.5 (1.1-2.8)	34.4 (27.4-40.3)	4.5 (2.7-6.5)	4.9 (3.8-6.6)	30.7 (27.5-36.0)	10.0 (7.5-14.9)
3. Corn Oil	—	—	—	12.4	—	1.4	26.7	59.4
Serum chylomicra								
(a) controls								
Cream layer	0.5 (0-0.8)	0.7 (0-1.4)	0.2 (0-0.7)	14.0 (13.2-14.7)	0.7 (0.4-1.2)	1.9 (1.7-2.3)	26.3 (22.5-31.4)	55.7 (51.2-59.8)
Saline layer	1.3 (0.5-1.6)	2.5 (1.1-2.9)	1.0 (0.5-2.6)	31.2 (28.4-36.5)	2.2 (1.4-3.2)	6.3 (3.9-8.4)	27.3 (23.4-28.3)	28.2 (21.0-31.1)
Serum chylomicra								
(b) hyperlipemics								
Cream layer	0.5 (0.3-0.6)	1.1 (0.6-1.8)	—	19.0 (15.3-22.0)	1.3 (1.0-2.5)	2.6 (1.9-3.1)	29.7 (26.4-36.7)	45.9 (40.8-57.2)
Saline layer	1.1 (0.8-2.8)	2.9 (1.3-4.5)	1.3 (0.9-2.3)	35.8 (27.7-39.2)	4.3 (3.4-7.3)	7.6 (5.0-12.3)	28.5 (21.1-41.5)	18.5 (16.4-26.1)

During a period of unrestricted dietary fat intake, the fasting serum of hyperlipemic patients is rich in chylomicrons of various densities; and it is particularly rich in the very lowest density particles, which are concentrated in the "cream" layer following centrifugation. The fatty acid pattern of this "cream" layer was found to simulate closely that of alimentary lipemia, produced by the ingestion of a meal rich in animal fats. Restriction in the dietary intake of animal fats in these patients resulted in the clearing

of the lipemia, a decrease in the volume of the very light cream layer of chylomicrons, and a change in its fatty acid composition (a relative decrease in C18:1 and relative increase in C16:0, C18:0 and C18:2 acids). Conversely, following the administration of cream and different oils, the serum fatty acid compositions in the "cream" layers of normal and hyperlipemic subjects became practically identical with that of those fed fats. These findings indicate that under ordinary circumstances dietary fat is the chief source of



the very light serum chylomicron fraction in both normal and hyperlipemic subjects.

The relative similarity of the fatty acid patterns of the fasting serum chylomicrons and the very lowest density lipoproteins of normal subjects and hyperlipemic patients following the clearing of their lipemias, is suggestive of a common origin of these serum lipids, for example, lipogenesis from carbohydrate and other nonfat substances. The few minor differences in fatty acid composition observed among them can be explained by the extent to which these fractions are contaminated by the very light chylomicrons of exogenous origin.

Depot fat represents still another source of serum lipids.<sup>25</sup> The mobilized fat appears to have a tendency to distribute itself among the various serum chylomicron fractions. Therefore, when fat mobilization from the depot is accelerated, as by the use of rice diet with restricted calories, and starvation due to severe malabsorption, the fatty acid patterns of the various serum chylomicron fractions are found to be similar to one another. The patterns of all these fractions are in turn similar to that of the depot fat.

Thus, serum chylomicrons may represent mixtures of triglycerides of three origins: diet, fat depot, and synthesized fat. Data obtained from the present study indicate that dietary fat is chiefly responsible for the lipemia in this series of patients. Also, with almost total elimination of fat from the diet, the lipemia may be maintained, at least temporarily, by accelerated mobilization of fat from the fat depot.

Several mechanisms for hyperlipemia, mainly related to defective removal of triglycerides from the blood, have been reported.<sup>26-30</sup> In view of the demonstration in patients in this series of a prolonged and increasing alimentary lipemia, together with

Figure 5

*Changes in the mean percentage fatty acid composition of very light serum chylomicrons (hollow bars) and the saline floatable heavier density particles (solid bars) before and after cream, coconut oil, and corn oil feedings in 10 hyperlipemic patients.*

a progressive change of the plasma fatty acid pattern in the direction of that of the ingested fat, an abnormality in the mechanism in gastrointestinal absorption of fat might be considered as an additional factor in the production of hyperlipemia. This possibility is now being actively investigated in this laboratory.

In analyzing the serum fatty pattern in patients with coronary artery disease, James and his associates<sup>24</sup> found a perceptibly high oleic acid content and a greater oleic/stearic ratio in their acetone-soluble plasma neutral fat fraction than in the matched normal controls. Because the serum chylomicrons were separated by chemical means and not by ultracentrifugation, no direct comparison can be made with the findings obtained in the present study. Nevertheless, the same findings as described by James et al. were observed in this series of patients, when they had either an absolute or a relative increase in the very light chylomicrons in their fasting sera. The possible correlation between the very light serum chylomicron fraction and its fatty acid composition with coronary artery disease deserves further and more thorough investigation.

#### Summary

The fatty acid patterns of the serum chylomicrons of 10 normal and 10 hyperlipemic subjects were determined by gas-liquid chromatography, before and after feedings of cream, corn oil, and coconut oil. Their depot fat composition was analyzed. Changes in the composition of the fasting serum chylomicrons of these hyperlipemic patients after they were stabilized on low fat, and rice and fruit diets were also studied.

Upon a full American diet, after 12 hours of fasting, the fatty acid pattern of the very light serum chylomicrons of the hyperlipemic patients was found to reflect that of the ingested fats, consisting chiefly of a mixture of saturated animal fats. The effect of food fats was less evident in the heavier serum chylomicrons and very low density lipoprotein fraction of these patients, where

the pattern of endogenously synthesized fat predominated.

A low-fat diet in eight of these patients caused a significant lowering of the serum chylomicron (triglyceride) concentration and a shift of chromatographic pattern of the very light serum chylomicrons toward that of the non-hyperlipemic subjects. It is apparent that the clearing of the exogenous fats from the serum permits the endogenous lipids to dominate the picture.

A low caloric rice and fruit diet appeared to arouse a temporary acceleration in the mobilization of fat from the body depot in two patients. In these patients, the fatty acid composition of serum chylomicrons, the very low density lipoproteins, and the depot fat were found to be similar to one another.

Dietary fat contributed directly to alimentary lipemia of the normal and diseased subjects. At or near the peak of alimentary lipemia, the composition of the very light serum chylomicrons of all subjects was found to be practically identical with the ingested fat. Alimentary lipemia of hyperlipemic patients was intensified and prolonged.

A relative increase in oleic acid, together with an elevated oleic/stearic ratio has been reported in the serum chylomicrons of patients with coronary artery disease. The same findings occurred in our patients, when there was an increase (relative or absolute) in the very light serum chylomicrons, due either to excessive dietary fat intake or to increased mobilization of fat from the depot.

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# The Architecture of the Right Ventricular Outflow Tract in the Normal Human Heart and in the Presence of Ventricular Septal Defects

By ROBERT P. GRANT, M.D., FRED M. DOWNEY, M.D.,  
AND HUGH MACMAHON, M.D.

**A** DETAILED UNDERSTANDING of the outflow tract of the right ventricle is needed for further progress in the diagnosis and surgical treatment of cono-truncal cardiac anomalies. Previous studies of the musculature in this region overlooked or undervalued its intrinsic muscular system and considered the bulbar musculature simply a part of the over-all muscular bundles of the two ventricles.<sup>1, 2</sup> This may have been because the authors did not take into consideration evidence that the musculature of the right ventricular outflow tract has a different embryologic origin than has the remaining musculature of the ventricles.<sup>3, 4</sup>

The present studies are based upon careful dissections of the outflow tracts in normal and congenitally abnormal human hearts. The studies affirm the existence of an intrinsic bulbar musculature which appears to be only secondarily coupled to the remaining ventricular musculature and may be different from it in still other regards. In addition, the studies shed light on the morphogenesis of outflow tract anomalies and point to a different theory of the manner in which ventricular septal defects and infundibular stenosis develop than has prevailed in the past.

## Nomenclature

Although the heart is a three-dimensional structure, pathology nomenclature and methods for study are essentially one-dimensional. Structures are named because they protrude, indent, or marginate rather than because of a relationship to over-all functional structure.

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While this is not the place to reorient cardiac anatomic nomenclature, it is necessary for the purposes of this study that certain "place names" of cardiac morphology be accurately defined.

### Crista Supraventricularis

This term has been used for widely diverse components of the outflow tract. Monckeberg, Abbott, and others had defined it as the portion of the free wall of the right ventricle that vaults the outflow tract; the musculature of the septum plays no part in this definition.<sup>5-7</sup> More recent authors have followed Keith<sup>8</sup> in fitting the term to embryologic theory. They consider the crista to consist of two muscle bundles on the septal surface of the outflow tract, the "septal" and the "parietal" bundles, which are believed to be derived from the two muscular ridges of the fetal bulbus cordis.<sup>9-11</sup> And still other authors use the term to define a horizontal ridge low on the septal surface that separates the inflow from outflow portions of the right ventricle.

The term crista supraventricularis was introduced by the French anatomist Wolff in 1791.<sup>12</sup> He used it simply to describe the mass of right ventricular tissue that lies between the tricuspid and pulmonary rings without regard to the muscular components it comprised or its embryologic origin. In this usage, it belongs to the free wall of the right ventricle, forming the medial wall of the outflow tract, and against which the aorta curls at its root. Indeed, prior to Wolff, this region had been called the "aortic wall" and the "fleshy pons" of the right ventricle. Wolff likened it to a spur ("éperon"). In translating it into Latin, spur becomes crista; and perhaps this is where the confusion developed, for the English language is readier to translate

erista as "crest" than "spur," and the crista supraventricularis has been treated by English and American writers as a crest or ridge on the septal surface of the heart, and not as a spur. Nevertheless, Wolff recognized that the muscle mass between the two orifices of the right ventricle is unique, having no parallel in the left ventricle, and he felt it deserved separate designation. In the present study we shall use Wolff's definition.

#### **Moderator Band and Trabecula Septomarginalis**

As early as da Vinci, anatomists had noted a free band of muscle extending from the septal surface to the free wall of the right ventricle. The anterior papillary muscle, which supports the anterior leaflet of the tricuspid valve, usually originates from it. In 1837 King<sup>13</sup> gave it the name "moderator band" as a result of his conjecture that it might control the capacity of the right ventricle as a sort of governor, permitting dilatation when too much blood might surge into it. On the septal surface the moderator band is continuous with a ridge of musculature originating at the membranaceous septum. This entire muscle structure including the moderator band was named "trabecula septomarginalis" by Tandler, and he suggested that the moderator band was simply that portion of this muscle mass that emerged from the septal surface to extend into the free wall of the right ventricle. With fine Gallic diffidence French anatomists have called Tandler's trabecula "le faisceau innominé." Certain writers have occasionally included the trabecula within their definition of a crista supraventricularis but this does not appear to enhance the usefulness of either term. As will be seen, separate identification of the trabecula septomarginalis may be useful, since this probably identifies the most caudal contribution of the bulbus cordis to the ventricular myocardium. On the other hand, the myocardial fibers of the moderator band are continuous not only with those of the trabecula septomarginalis but with other muscular components of the outflow tract, and the presence or absence of the moderator band and its size in hearts with ventricular septal defects is often

a useful lead in studying the bulbar musculature.

#### **Muscle Bundles of the Ventricles**

Since the efforts of Richard Lower in the seventeenth century to unroll the musculature of the heart there have been countless studies of the directional arrays of the fibers of the myocardium. In certain species of animals it appears to be possible to develop what appear to be cleavage planes between these arrays. Such seemingly independent directional arrays are called "bundles." The bundles of the left ventricle have been studied in many different species. The extent to which these bundles are morphologically distinct from one another has been the subject of controversy for many years.<sup>14</sup> The embryogenesis of myocardium is such that it is extremely unlikely that in any mammalian species the bundles are completely separate;<sup>15</sup> all bundles have fibers in continuity with fibers of adjacent bundles and species vary mainly in how numerous these bridges are. In our experience and that of others in recent years,<sup>16, 17</sup> it is quite apparent that in man the ventricular myocardium consists of the same directional arrays as in other mammals, but fibers of continuity and fibers with transitional directions are so frequent that no true cleavage planes exist. Nevertheless it is possible to separate individual directional arrays by sundering these points of continuity. In short, grossly the human ventricular myocardium appears to be genuinely a muscular syncytium, and the term "bundle" identifies a directional component of myocardial fibers rather than a discrete and independent group of fibers. For this reason the term "muscle component" is used instead of "muscle bundle" in the present study.

#### **Methods of Study**

In order to study the directions of myocardial fibers, the fibers must be rendered separable but with enough tensile strength that they will not tear easily. No ideal method for doing this has yet been devised. After experimenting with a number of tanning and other methods, none appeared to be superior to the general method originally used by Lower and later by MacCallum and by Mall.<sup>2</sup> The heart is immersed in water acidified with acetic

acid and simmered just below the boiling point for 3 to 4 hours. This removes much of the fat and softens connective tissue. At this stage, further fat and other connective-tissue structures including valvular and endocardial tissues are easily removed mechanically. Then, to restore the tensile strength of the fibers, the heart is carried through increasing concentrations of ethyl alcohol, with final dehydration for 3 to 4 days in absolute alcohol. Dissection is best done under a dissecting microscope. One must be cautious, however, not to dry the specimen under hot illumination, for the fibers will become tough and brittle.

Detailed dissections of the outflow tract of the right ventricle were performed on 15 dog hearts and seven normal human hearts. The hearts in six cases of ventricular septal defect were dissected completely, and partially in four others, including two cases of tricuspid arteriosus. Twenty-two additional human hearts with cono-truncal abnormalities from various sources were studied without dissection of the musculature, but with landmarks developed from the dissection as guides. In the present study the musculature of the septal region of the right ventricle and of the crista supraventricularis alone was studied, and no effort was made to study the architecture of the free wall. It is recognized that the number of heart studies is small and makes it impossible to develop a complete and secure picture of the architectural abnormalities of the cono-truncal anomalies, so that this must be viewed as a preliminary study.

A word about the embryogenesis of the interventricular septum may be appropriate for understanding these studies. It has been known for more than a century that two different muscular tissues join to form the septum.<sup>4</sup> One, growing from below, arises as an invaginating septum at the apex of the ventricular loop. It is often referred to as the muscular part of the interventricular septum. The other, growing from above, is an extension into the outflow tract (the bulbus cordis) of a septum that spirals down the truncus arteriosus to divide it into a pulmonary artery and an aorta. In the truncus, this septation is fibrous. Its extension into the bulbus, following the two bulbar ridges, is muscular and is called the bulbar part of the interventricular septum. Thus, the closing of the interventricular septum depends upon these two muscular septa, each derived from relatively opposite ends of the ventricular loop, meeting, overlapping, and fusing.

In studying the final stages of septal closure, embryologists have been most interested in the formation of the membranaceous septum. But this is only one place where the two tissues meet, and in the adult heart it is a relatively small region of the zone of fusion. The pathway of fusion ex-

tends from the membranaceous septum laterally to the general region of the moderator band. Then, since bulbar musculature is found only on the right ventricular surface of the interventricular septum, there is a large area of fusion of the two tissues where the invaginating septum grows over the posterior surface of the bulbar musculature to form the outflow tract of the left ventricle. While the sequence of events leading up to fusion of the membranaceous septum have been extensively and repeatedly studied, there appears to have been no study of the events leading to the fusion elsewhere of the two tissues.

### Results

#### The Normal Right Ventricular Outflow Tract

The septal surface of the right ventricular outflow tract normally has the dimensions of an isosceles triangle. The three apices of this triangle are the midpoint of the base of the posterior cusp of the pulmonic valve, the point where the moderator band emerges from the septal surface, and the point where the tricuspid ring crosses the membranaceous septum. Normally the three points are relatively equidistant from one another. This triangle in turn is congruent with a larger triangle representing the entire right ventricular septal surface. The relationship between the two triangles is useful in studying distributions of hypertrophy and dilatation in the right ventricle. Normally the distance from the posterior pulmonic valve to the membranaceous septum is relatively equal to the distance from the membranaceous septum to the posterior sulcus of the heart, and these two dimensions span the flow path in the right ventricle.

In figures 1A, B, and C are shown schematically the major directional components of the normal human bulbar musculature and their relationships to tricuspid, pulmonic, and aortic orifices and to left ventricular musculature. There are no discrete, isolatable fiber masses as the figures would suggest, but many gradations of fiber direction and abundant fiber continuities among these components. The schemata are to be viewed as graphs, demonstrating the major but by no means the only directional components of right ventricular outflow musculature.

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that "the muscle bundles of the conus form relatively simple rings which attach themselves to the root of the aorta," and Tandler,<sup>1</sup> the most thorough student of cardiac musculature, considered there was too much individual variation to permit detailed description. Neither of these views is correct. All components shown in the diagrams have been identified in every normal human and dog heart studied, and there is remarkably little variation in their directions and relationships from heart to heart.

In general, there are two layers of muscular components that form the intrinsic musculature of the right ventricular outflow tract. The superficial components are more complex and tend to have superior-inferior directions with their major mechanical effect apparently to shorten the outflow tract. The deeper layer, on the other hand, is simpler and has a horizontal direction, which, on shortening, would narrow the outflow tract.

The superficial layer consists essentially of three components (fig. 1A). They are best studied by first identifying the posterior cusp of the pulmonic valve for, at the midpoint of its base, can be seen a crease that separates two important components in this layer. (The crease is often best seen by first peeling off the fibrous sheet of endocardium overlying it; Keith<sup>8</sup> called this crease the infundibular raphe.) Lev<sup>9</sup> has named the two components the septal bundle (inserting on the left side of the cusp) and the parietal bundle (inserting on the right side of the cusp). From the embryologic data of Kramer,<sup>4</sup> Kjellberg<sup>10</sup> and Lev<sup>9</sup> have suggested that these two components are developed from two bulbar ridges, extensions of the ridges in the fetal trunus arteriosus which fuse to divide the trunus into the aorta and pulmonary artery. In hearts with trunus arteriosus, however, we have been able to identify a component in the ventricular wall having the location of the normal septal component and leading to a small moderator band, indicating that the septal component may develop normally even when the muscle ridges of the trunus are absent. Furthermore, in cases of transposition

where the lie of the ridge is presumably markedly abnormal and perhaps reversed, the septal component with its moderator band contribution can often be seen to be in normal position. While the muscular ridges undoubtedly make important contributions to bulbar musculature, there are so many components in this region that it is probably unwise to ascribe particular ones to specific fetal structures until more is known about cardiac morphogenesis.

A third component of the superficial layer of bulbar musculature is one that inserts on the right side of the pulmonic ring, descends within the crista supraventricularis to course obliquely across the septum, and contributes a major part of the fibers of the moderator band. It can be called the oblique component (component 3 in fig. 1A). It has not been previously described, perhaps because it is somewhat hidden by the parietal component, which it often passes under, or interweaves through, or, less commonly, passes over in its course.

The moderator band is an exceedingly useful structure in interpreting the bulbar musculature. Even with the most painstaking dissection it has been impossible to identify the proportion of its fibers that are derived from each bulbar component. The oblique component appears to make the largest contribution, and contributions from the deeper component may be only secondary syncytial fusing. In the present series of cases, hearts with ventricular septal defects due to absence of the oblique component had no moderator band, or at most a very slender structure derived entirely from the septal component. Absence of the moderator band, whether or not associated with a ventricular septal defect, should be viewed as a congenital anomaly of cardiac musculature for it represents a disturbance in the joining of bulbar and ventricular components of the right ventricle.

Beneath the superficial muscular components of the bulbus is a deeper layer of musculature much less varied in direction (fig. 1B). It originates on the membranaceous septum and aortic-pulmonary tendon, sweeps laterally at right angles to the direction of

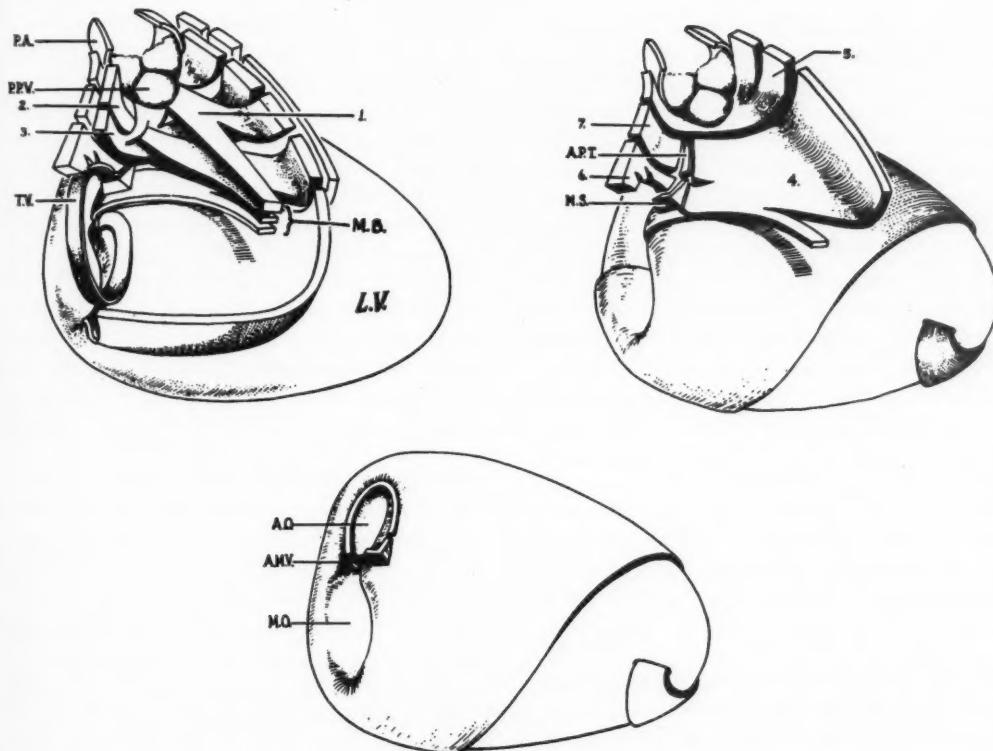


Figure 1

*Schema of directional components of right ventricular septal musculature. The heart is shown from the frontal view as it lies in the chest, with the free wall of the right ventricle removed. Upper left, superficial bulbar muscular components. Upper right, deeper bulbar muscular components; the superficial components have been removed. Bottom, all right ventricular components have been removed to show the left ventricular orifices and the sino-spiral muscle component. The major right ventricular muscular components are numbered: 1 is also called the "septal" component, 2 the "oblique" component, and 3 the "parietal" component. A.M.V., anterior mitral valve leaflet joining the two left ventricular trigones; A.P.T., aortico-pulmonary tendon; A.O., aortic orifice; M.B., moderator band; M.O., mitral orifice; M.S., membranaceous septum; P.A., pulmonary artery; P.P.V., posterior pulmonary valve; T.V., tricuspid valve fibrous ring.*

flow in the right ventricle, and curls anteriorly at the lateral margin of the right ventricle to merge in the right ventricular free wall, some portions also becoming continuous with the outermost layers of left ventricular musculature. The most inferior part of this layer originates from the membranaceous septum and contributes fibers to the moderator band. Cephalad is a thicker part originating from the aortic-pulmonary tendon. This tendon is a fibrous ring at the root of the aorta

and a major site of insertion of left ventricular muscle. The tendon originates from the membranaceous septum and the right (anterior) trigone of the left ventricle; it meets a similar fibrous ring at the root of the pulmonary artery, and it inserts in the left (posterior) trigone (fig. 1C). Farther cephalad the deep bulbar musculature encircles the right ventricle immediately below the pulmonic ring, passing over the aortic-pulmonary tendon, where the latter extends posteriorly.

Beneath this deep bulbar layer is the superficial sino-spiral muscle bundle of the left ventricle (fig. 1C), but there is no cleavage plane between the two layers, and myocardial continuity is as evident here as elsewhere in the heart. Nevertheless, it is interesting that the septal branch of the left coronary artery, which arises from its parent artery immediately behind the pulmonary artery, tends to run most of its course in or near a plane separating the deep bulbar and the ventricular musculature, emphasizing the developmental independence of the two.

The structures that develop from the path of junction between the bulbar and ventricular musculature are of especial importance, for here will be encountered developmental anomalies whenever there is a disturbance in differentiation of bulbar or ventricular parts of the heart. The moderator band and the membranaceous septum are such junctional structures and have already been mentioned. The septal leaflet of the tricuspid valve also depends developmentally upon both bulbar and ventricular musculature and is often deformed in anomalies of this region. The horizontal ridge of musculature extending from the membranaceous septum to the moderator band, called the crista septomarginalis, is also a junctional structure; it is formed in the main from bulbar musculature, especially deep components and parts of the oblique component. This ridge is an especially useful landmark because it is usually easily identified and it serves to demarcate inflow from outflow tracts, and bulbar from ventricular musculature. Another junctional structure is the septal papillary muscle, also called Lanensi's papillary muscle, which subtends chordae tendineae of the anterior leaflet of the tricuspid valve. This is the only papillary muscle of the tricuspid valve that is derived entirely from bulbar musculature. Anatomically, it emerges from the septal surface of the right ventricle low in the outflow tract about midway between medial and lateral walls, but its muscle fibers are derived from the septal component of bulbar musculature, with some fibers coming from the oblique component. Here then is a structure that, from

the origin of its muscle fibers to the insertion of its chordae, spans the diameter of the outflow tract, and for half of this distance lies free in the outflow stream of the right ventricular chamber. As a result, the size of the papillary muscle and the degree of its displacement from the septal component, where its fibers originate, may be gauges of the type and amount of hemodynamic stress in the outflow tract during development. Structures such as this offer ready, simple elements for approaching the architecture of the heart from a semi-quantitative point of view. Needless to add, abnormalities of this papillary muscle and its chordae are related to defects in fusion of ventricular and bulbar musculature and are often associated with malformation of the septal leaflet of the tricuspid valve.

#### **Muscular Architecture of Ventricular Septal Defects**

Congenital defects of the ventricular septum are of three general types according to location. 1. Defects at the A-V ring such as are seen in association with persistence of the ostium primum of the atrial septum and with A-V cushion defects. Almost certainly these defects are partly at least due to a fusion failure between bulbar and muscular septa, for they lie adjacent to the A-V ring beneath the junction of the septal and posterior leaflets of the tricuspid valve, for this is where the two septa meet. 2. Defects in the main body of the inflow tract of the right ventricle, the "muscular" septal defects. These are often circuitous, sinus-like tracts through the septum, margined by well-formed trabeculae carneae, and they usually are unassociated with other cardiac malformations. 3. Defects in the outflow tract of the right ventricle, the "bulbar" septal defects. These may lie anywhere in the triangle formed by the outflow tract and are frequently associated with other anomalies of the bulbar and truncal regions of the heart. They tend to be circular, smooth-edged, gaping holes communicating between the two ventricular chambers.

The difference in morphology between muscular and bulbar septal defects suggests that there may be a difference in their manner of development. At early fetal stages, the myocardium is a spongy sinusoidal mass, and it

is not difficult to visualize the "muscular" septal defect as representing persistence of a type of sinus tract, where sinusoidal elements failed to be obliterated during later condensation of the myocardium.<sup>3</sup> On the other hand, the round gaping character of the bulbar septal defect suggests that it is the result of failure of certain muscular components of the bulbus ever to develop, that a discrete part of bulbar musculature is absent.

This proves to be the case. When the hearts with bulbar defects were dissected with the schemata of figure 1 as a guide to normal musculature, in all hearts one or more directional components was found to be absent. The location of the defect in the outflow tract proved to be a function of the particular muscular component or components that had failed to develop, and the remaining musculature of the bulbus consisted of directional components that could be related to those present in the normal heart. This was especially easy to demonstrate in hearts with single, small defects. When the defect was large and associated with other complex outflow derangements, it was more difficult to be confident that the remaining bulbar musculature represented components present in the normal heart. On the other hand, it was in these hearts with extensive defects that the absence of specific components was most obvious. For example, in cases of persisting trunus arteriosus or so-called single ventricle, often there was only a single muscular component ridging the region where bulbar musculature would have been present. Usually it resembled the septal component in its origin and distribution, and no other bulbar musculature could be identified.

Kjellberg and his associates<sup>10</sup> have suggested that in hearts with extensive defects the derangement may be due to displacement of one or both of the two bulbar ridges mentioned earlier that lie at opposite points on a diameter across the bulbus and are extensions of the spiraling trunical ridges that form the trunical septum. This hypothesis is derived in part from the once widely held notion that unequal size of pulmonary artery and aorta in congenital heart disease is due to eccentric

growth of the trunical septum. Shaner,<sup>18</sup> however, has offered convincing evidence that in most instances such inequality is a consequence of a hemodynamic abnormality resulting from the congenital lesion and not to eccentric septation. Among cases in the present study in which eccentric septation might have been expected, as in pulmonary atresia, structures known to be derived from the bulbar ridges, such as the membranaceous septum, were normally located. While the hypothesis of Kjellberg and his associates is plausible, in none of the hearts studied in this series was it needed to explain the bulbar musculature derangement.

A muscular defect communicating between right and left ventricles involves three layers of septal musculature, two bulbar and one ventricular, and the architectural defect at all three levels must be examined. As far as the superficial bulbar layer is concerned, after familiarity with the musculature by dissecting normal and abnormal hearts was acquired, it became possible to identify the absent component readily from the surface topography alone, without dissection. The deep bulbar layer was more difficult to evaluate because, unlike the superficial components, only part of a deep component might be absent in a given case. While the missing component in the superficial layer governed where the defect would lie in the outflow tract, the missing deeper component seemed to determine how big the defect would be and whether or not the foundation of the aortic root would be disturbed (i.e., whether or not "over-riding" or dextro-position of the aorta would be present).

On the other hand, at the third level (the left ventricular part of the septum), no specific bundle or component defect could be identified to indicate a basic architectural defect of this layer. The left ventricular fibers ramified and otherwise increased their numbers on each side of the defect, so that the general density, directions, and distribution of fibers in the left ventricular layers of the septum appeared to be unaltered by the defect.

This observation indicates that bulbar sep-

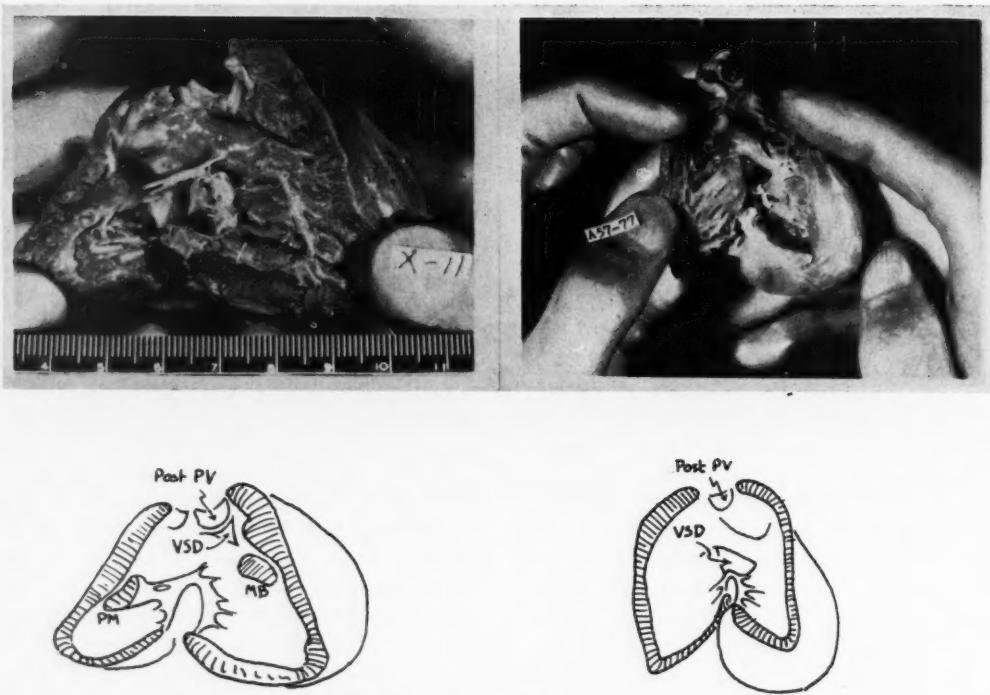


Figure 2

Two hearts with ventricular septal defects, with diagrams. Left, the defect is subvalvular and the moderator band is prominent. Right, only the parietal component is present bridging a large defect; there is no moderator band.

tal defects are primarily disturbances in growth and differentiating of bulbar musculature and only secondarily of ventricular musculature. Evidently, in the formation of the interventricular septum, the part invaginating from the apex of the ventricular loop grows upward until it meets the bulbar musculature, and it can only grow beyond this point when there is intact bulbar musculature over which to grow. When there is a defect in the bulbar musculature this invaginating tissue cannot grow across the defect but will grow around it. No heart has yet been seen by us or described in the literature in which bulbar musculature was absent and the septum was closed by ventricular tissue alone. This suggests that the bulbar musculature plays the role of an "organizer" tissue for ventricular muscle, in the embryologist's meaning of the term. Furthermore, it raises the possibility of other fundamental anatomic

and biochemical differences between the two types of cardiac musculature, each derived from opposite ends of the primitive cardiac tube. In other studies, for example, preliminary findings suggest that the two musculatures may undergo hypertrophy differently.<sup>10</sup>

If the bulbar abnormality is confined to the superficial layers alone, and deeper bulbar layers are intact, there will be a bulbar derangement but no through-and-through septal defect. In a heart with absence of the moderator band, this proved to be the case, for there was no musculature having the direction of the oblique component. Absence of the moderator band or of the septal papillary muscle should be viewed as congenital cardiac defects even though no hemodynamic abnormality is produced.

It was mentioned that the particular location of a bulbar septal defect depends upon the location of the missing superficial bulbar

muscular component. To illustrate this, if the septal component and the high deep component are missing, the defect will be subvalvular in location. The case in figure 2 (*left*) is an example of this. The moderator band is present, indicating that the septal component is not a major contributor to this structure. If, on the other hand, the oblique and low deep components are absent, the defect will be adjacent to (and might include) the membranaceous septum. If *both* the septal and oblique components are missing, the heart illustrated in figure 2 (*right*) will result. Here, the only remaining bulbar muscle is the parietal bundle and its horizontal division extending from the crista supraventricularis to the lateral wall of the outflow tract. No moderator band is present. The entire width of the low deep component is absent. Although there seem to be two bulbar septal defects in this heart, actually it is only the bridging by the parietal component, the sole remaining major septal component, which divides the defect into two holes.

Earlier it was mentioned that the presence or absence of the deep bulbar component governs the relationship of the aortic root to the septal defect. As shown in figure 1, the deep component inserts on the aortico-pulmonary tendon at the root of the aorta immediately proximal to the aortic cusps. If this muscular layer is absent (combined with the fact that left ventricular tissue cannot grow where there is no bulbar musculature) the anterior lip of the aorta will no longer have an attachment to the left ventricle, and will be supported solely by musculature of the free wall of the right ventricle (components 5, 6, and 7 of figure 1B). As a result, the right ventricular chamber will open directly into the root of the aorta. This appears to be the anatomic explanation for over-riding or dextro-position of the aorta in hearts with ventricular septal defects.

In the past, over-riding has been considered to be due to an abnormal position of the aortic root in the skeleton of the heart. Edwards<sup>20</sup> and Schoenmackers<sup>21</sup> pointed out that this was probably not the case, and suggested that it was due to the fact that the

aorta at its root curls sharply anteriorly against the crista supraventricularis. A defect high in this region would indeed "look" into the root of the aorta. More direct proof that there is no abnormality of the skeleton of the heart in this disorder was obtained in the course of other studies that will be reported in greater detail elsewhere.<sup>22</sup> Fine silver wire was threaded along the rings of all four ventricular orifices (mitral, tricuspid, aortic, and pulmonic) and along certain other ventricular landmarks in a series of normal hearts and hearts with various cono-truncal abnormalities including over-riding aorta. Roentgenograms in two planes at right angles to each other were obtained for each specimen. By use of methods of descriptive geometry, three-dimensional measurements were made of the location of the orifices in relation to each other and to other ventricular structures. In all cases of over-riding aorta the aortic orifice was in completely normal position with respect to the skeleton of the heart.

What is the architectural basis for the muscular stenosis so frequently seen in the infundibulum in hearts with bulbar septal defects? This is a much more difficult problem to study and will require extensive detailed fiber counts and measurements among the bulbar muscular components in normal and abnormal hearts before the answer can be given confidently. From the gross method of dissections used in the present study, in these hearts at least, the stenosis was due to selective hyperplasia of individual bulbar muscular components, and the particular location of the hypertrophied component or components determined where in the outflow tract the stenosis would occur. For example hypertrophy of the septal component alone cannot obstruct outflow. When the septal component is hypertrophied along with the parietal component, the stenosis will be immediately subvalvular. If there is no accompanying hypertrophy of the septal component, a small infundibular chamber between the site of obstruction and the pulmonic valve will result. On the other hand if the oblique component is hypertrophied, the obstruction will lie much lower in the outflow tract, adjacent to the

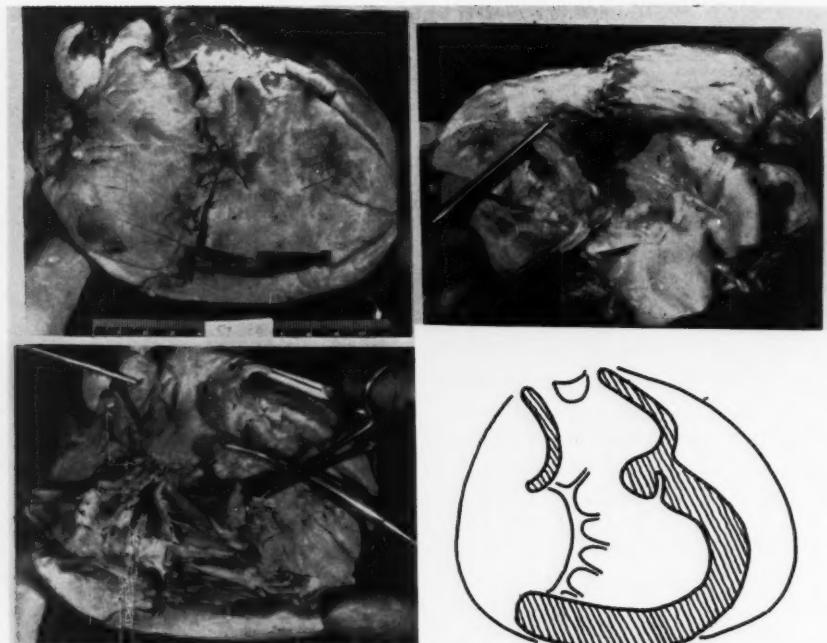


Figure 3

*Infundibular stenosis low in the outflow tract due to hyperplasia of the oblique component.* Upper left, the reconstructed heart viewed frontally as it lay in the chest. Upper right, the right ventricular chamber viewed from above through the infundibulum. The marked narrowing of the chamber at mid-position is seen. Below, the free wall of the right ventricle is reflected for a frontal view of the right ventricular septal surface, which is shown in the diagram at right; the cut surface of right ventricular free wall is shaded.

moderator band. The heart in figure 3 illustrates this, an example of a "three-ventricle" heart.

These findings suggest that infundibular stenosis is a basic part of the growth abnormality in ventricular septal defects rather than a secondary adaptation of the heart to the hemodynamic abnormality produced by the septal defect. It has been pointed out that injury to pre-differentiated tissue can result in either overgrowth or arrest of growth of the tissue.<sup>3</sup> From the present studies it is suggested that in these bulbar syndromes injury to pre-differentiated bulbar primordia results in overgrowth of the bulbar muscular component in some hearts (infundibular stenosis without a septal defect); in other hearts certain components are arrested while other components overgrow (ventricular septal de-

fect with infundibular stenosis); and in still other hearts arrest of growth alone takes place (simple ventricular septal defect if both layers of bulbar musculature are involved; topographic disturbances, such as absence of the moderator band without a transseptal defect, if only the superficial layer is involved). Thus it seems a likely hypothesis that the ventricular septal defect and the stenosis are both due to the same primordial injury in a given case, and whether hypertrophy, or defect, or both will occur is, perhaps, a function of the severity or timing of the damage to primordia of specific muscular components.

#### Discussion

While this study has been mostly concerned with the nature of outflow-tract architecture in normal and abnormal hearts, one of its re-

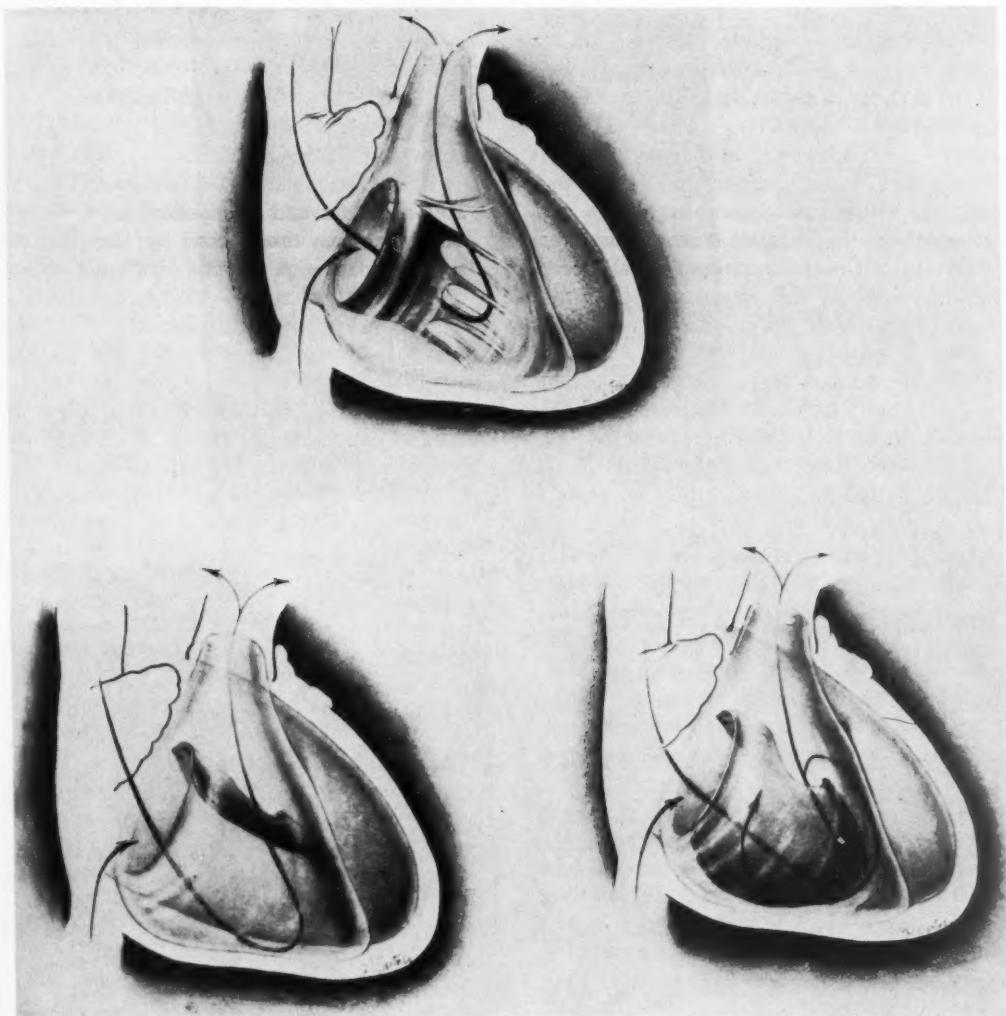


Figure 4

*Schema of the valvular abnormality in Ebstein's anomaly. The heart is viewed frontally as it lies in the chest, and the right ventricular free wall is made transparent. Arrows indicate pathways of blood flow. Above, the normal tricuspid valve; below, two degrees of malposition of tricuspid valvular tissue. The valve inserts on the septum along the path of fusion of bulbar and ventricular myocardium. The difference in degree of deformity is due to differences in the extent to which lower parts of the tricuspid valve leaflets are displaced.*

sults has been to direct attention to a region of the heart where congenital malformations occur that has not been greatly appreciated in the past. This is the zone of junction between bulbar and ventricular musculature. This is the zone dividing inflow from outflow

tracts and extends from the membranaceous septum and septal leaflet of the tricuspid valve on the medial side of the right ventricle to the region of emergence of the moderator band laterally. For example, the ventricular abnormalities that may be associated with per-

sistence of an ostium primum lie in this zone: the ventricular septal defect lies beneath the septal cusp of the tricuspid valve, the cleft in the anterior leaflet of the mitral valve runs up to the A-V ring at this same point, frequently there is a malformed septal leaflet of the tricuspid valve, aneurysm or multiple perforations of the membranaceous septum, etc., all lesions in this junctival zone. Almost certainly the left axis deviation seen electrocardiographically in this syndrome is another result of the faulty fusion of bulbar and ventricular septum. Evidently there is a disturbance in the development of the anterior division of the left bundle branch, for this division normally is distributed on the portion of left ventricular muscle that grows over the bulbar musculature to complete the interventricular septum.

Another syndrome that appears to be due in part at least to an abnormality at the junction of ventricular and bulbar musculature is Ebstein's anomaly. Here the line of attachment of the displaced septal and posterior rim of the tricuspid valve is near or within, but never beyond, this zone of junction from membranaceous septum to moderator band (fig. 4). The portion of the tricuspid valve which affixes to the membranaceous septum is often the only part normally attached. The only hearts with obliteration of the membranaceous septum by musculature that we have seen are two cases of Ebstein's anomaly. Goerttler, on the basis of embryologic studies, first suggested that Ebstein's anomaly might be due to a defect of growth during the stage of invagination of the ventricular loop to form the septum, and our observations give circumstantial evidence that tends to confirm this hypothesis.<sup>23</sup> With greater appreciation of the anatomy of this region, no doubt other developmental anomalies will be identified that are related to the manner in which bulbar and ventricular musculatures fuse to form the septum.

Another major purpose of this study has been to bring quantitative methods into the study of cardiac morphology. Understanding of cardiac function can never be complete until its morphology as a pump is also under-

stood. Progress in cardiac physiology has depended almost entirely upon developing methods for quantifying physiologic processes. To discover the quantifiable elements of the morphology of the heart is the challenge for cardiac pathologists. Since morphology is a problem in surfaces and spaces, the quantifiable elements and mathematical tools will be different from those used by the physiologist or the biochemist. In the outflow tract of the right ventricle, for example, the quantifiable elements prove not to be discrete muscle bundles, but directional properties of a densely syncytial musculature. The particular array shown in figure 1 is, then, more a graph than a picture of a muscular system. Furthermore, as in all graphs, it is a generalization and simplification in order to have a manageable schema upon which to begin to erect an understanding of the architecture of this region of the heart.

#### Conclusions

Detailed dissections of the musculature of the right ventricular outflow tract were performed in a number of normal and congenitally abnormal human hearts. In cases of bulbar ventricular septal defects the dissections disclosed absence of one or more components of bulbar musculature, with the remaining musculature made up of directional components that could be related to those seen in normal hearts. It is concluded that the bulbar ventricular septal defect is not due to failure of bulbar components to fuse, but to failure of certain muscular components ever to develop; and the particular location of the septal defect in the outflow tract is a function of the lie of component or components that failed to develop.

Over-riding or dextro-position of the aorta is shown to be not due to an abnormality of the location of the aorta with respect to the skeleton of the heart, but due to failure of certain deeper bulbar muscular components to develop. As a result, the septal edge of the aortic ring no longer attaches to left ventricular musculature, and therefore the aorta faces directly into the right ventricular chamber.

The muscular hypertrophy accounting for

infundibular stenosis also appeared to be confined to certain directional components of bulbar musculature, with actual hyperplasia of that component. The location of the stenosis in the outflow tract depended upon which component had undergone hyperplasia. It is suggested that in outflow tract anomalies, septal defects and the infundibular stenoses are both direct consequences of a primordial injury, and whether a given component fails to develop or undergoes hypertrophy may depend upon the severity or timing of the damage to that component.

Congenital anomalies of the right ventricular outflow musculature are of two types. 1. Defective development of intrinsic bulbar muscular components; ventricular septal defects and infundibular stenosis, whether or not associated with other anomalies, are examples of this. 2. Defects in the manner by which normally elaborated bulbar musculature joins and fuses with the invaginating ventricular septum to form a closed ventricular septum. Examples of this include the ventricular anomalies associated with persistent ostium primum and with A-V communis; other examples are Ebstein's anomaly, defects and aneurysms of the membranaceous septum, and absence of the moderator band. With greater awareness of this zone where ventricular and bulbar musculature meet, no doubt other examples of coupling anomalies will be discovered.

#### Acknowledgment

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## Retrograde Conduction to the Atria in Ventricular Tachycardia

By ALBERT D. KISTIN, M.D.

**C**ONTRARY to the prevalent view that retrograde conduction to the atria in ventricular tachycardia is rare,<sup>1</sup> the author has recorded tracings consistent with such conduction relatively frequently with simultaneous standard and esophageal leads. The standard electrocardiographic tracings often fail to demonstrate atrial activity accurately, because the atrial deflections are small and lost in the deflections of the ectopic ventricular activity. This is apparent from a comparison of lead II with the simultaneously recorded esophageal lead in most of the illustrations of this paper, and probably explains why so few cases of ventriculo-atrial (V-A) conduction in ventricular tachycardia have heretofore been recognized.

Sir Thomas Lewis described the first clinical case of ventricular tachycardia in 1909<sup>2</sup> and observed that in dogs retrograde conduction to the atria was common.<sup>3</sup> A review of the clinical literature by Foster and Thayer in 1950<sup>4</sup> yielded only three cases of 1:1 V-A conduction and six cases of V-A conduction with variable block. These authors concluded from the illustrations of 81 published cases of ventricular tachycardia that in 40 it was impossible to recognize the atrial activity. The interpretation of V-A conduction was made in standard electrocardiographic leads<sup>1, 5-26</sup> and recently in esophageal leads.<sup>25, 27-32</sup>

### Material and Methods

Ventricular tachycardia for the purposes of this study is defined as five or more ectopic ventricular

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systoles in succession. Simultaneous esophageal and standard leads<sup>33</sup> were recorded. During the period of study the interpretation of ventricular tachycardia was made in 21 cases, 14 in the course of clinical practice, five during cardiae catheterization, one during mitral valve surgery, and one during surgery for coarctation of the aorta.

Whenever the interpretation of ventricular tachycardia with 1:1 retrograde conduction to the atria was made, with one exception, the onset of one or more runs of tachycardia was recorded and consisted of a bizarre QRS different from the QRS of sinus origin. Parts of the tracing showing regular sinus rhythm were available for comparison. The esophageal leads ruled out the possibility of atrial tachycardia with aberrant conduction by demonstrating that the tachycardia did not start with a P wave. In the case in which the onset of the tachycardia was not observed, within a few beats after the cessation of the tachycardia isolated ventricular premature systoles with retrograde conduction to the atria were recorded identical in configuration with the complexes of the tachycardia.

In 10 earlier studies the esophageal electrode was paired with the Wilson V connection (VE lead). In 11 more recent studies a bipolar esophageal lead (BE lead) was recorded simultaneously with a standard lead, usually lead II, and two VE leads, one each from the electrodes of the BE lead as described by Copeland et al.<sup>32</sup> Instead of the fluid-filled tubes recommended by these authors a simple Rehfuss tube was used with two German-silver rings, 3.0 mm. wide and 2.0 cm. apart. The lower of the rings was about 3 cm. from the tip of the tube, and each ring was connected to insulated wires passing up the inside of the tube. The holes in the tip of the Rehfuss tube were sealed with solder to avoid wetting the wires. The simpler BE electrode yields satisfactory tracings.

The BE lead is often superior to the VE lead for the study of V-A conduction. It is usually possible to select an esophageal position for the electrodes at which the BE lead records small QRS complexes and large P waves, the latter distinct even in complex arrhythmias when superimposed on QRS and T (figs. 1 and 3-6). Also it is often possible to select an esophageal level at which the BE lead records retrograde P waves more or less opposite in direction to the sinus P waves (figs. 1 and 3-5); such tracings are ob-

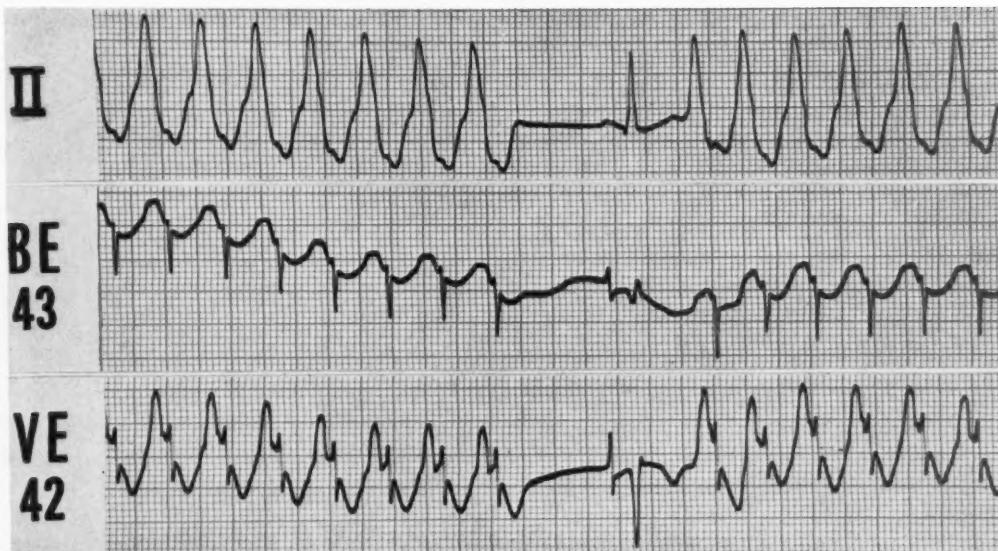


Figure 1

*Case 1. Simultaneous lead II, bipolar esophageal lead, and V esophageal lead. Ventricular tachycardia with 1:1 V-A conduction. One sinus beat interrupts the tachycardia. The retrograde P waves in the BE lead are the large spiked downward deflections after each small rounded QRS; the sinus P wave is smaller, biphasic, with a larger upward component. The retrograde P waves are distinct in the VE lead, but there is less difference from the sinus P wave than in the BE lead. A similar consecutive run of 228 beats was observed with no increase of V-A conduction time.*

tained with VE leads less frequently. Occasionally the retrograde conduction is clearer in a VE lead than in a BE lead.

The optimum position of the esophageal tube is determined by scanning tracings obtained from several levels. The position yielding the best P waves for study varies with individuals and with the contour and direction of QRS and T.

The illustrated electrocardiograms are recorded at a paper speed of 25 mm. per second on 2- or 4-channel direct-writing electrocardiographs (Sanborn). The smallest ruled interval is 0.04 second. The numbers under VE in the illustrations designate the position of the esophageal electrode in centimeters from the nares. The numbers under BE designate the midpoint between the two electrodes of the lead in centimeters from the nares. When no number follows VE or BE, the level of the electrodes was not recorded. The illustrated photographs of the tracings are not retouched.

#### Observations and Discussion

##### Incidence of V-A Conduction

Table 1 is a summary of the cases with V-A conduction. Of 21 cases in which the

interpretation of ventricular tachycardia was made 1:1 V-A conduction alone occurred in five. The 1:1 conduction often did not appear until the second beat of the tachycardia because of initial interference with the sinus beat, and then persisted to the end of the run. In five other cases one to many runs with 1:1 V-A conduction occurred in association with other runs in the same tracing with other atrial mechanisms. One of these cases was previously reported (case 7).<sup>27</sup> The other mechanisms were either independent atrial rhythm or irregular V-A conduction including the Wenckebach phenomenon. In four cases V-A conduction occurred irregularly and there were no runs with 1:1 V-A conduction; in 3 cases there were also runs with independent atrial rhythm in the same tracing, and in 1 case runs that demonstrated the Wenckebach phenomenon. In only 7 of the 21 cases was the atrial mechanism always

Table 1  
Ventricular Tachycardia with Ventriculo-Atrial Conduction

Patient no., age (yr.) (fig.)	Diagnosis	Digitalis in 4 weeks preceding tracing	Longest recorded uninter- rupted run No. beats (or min.)	Average rate in ventricular tachycardia (per min.)	Sinus rate per min.	P-R (sec.)	X-P* (sec.)	Atrial mechanism	Evidence for ventricular focus
1 M/69 (fig. 1)	MI (old), PE	No	228	88	186	0.15	0.17	1:1 V-A	APS
2 M/68 (fig. 7)	HAD, PE, CWP	Yes	23	103	194	0.15	0.12	1:1 V-A	
3 M/16	CA (Surg)	No	5	76	94	0.14	0.18	1:1 V-A	—
4 M/64 (fig. 8)	AHD, DM, PE	Yes	11	75	107*	0.15	0.14	1:1 V-A	QRS form
5 F/42	MS (Surg)	Yes	6	50	91	0.17	0.18	1:1 V-A	APS
6 F/43 (fig. 2)	AS, No HD	No	7	92	182	0.16	0.20	1:1 V-A + Irregular V-A. (Wenckebach)	Reciprocal beats
7 M/36 (bibl. #27)	AS, No HD	No	40	122	207	0.13	0.12	1:1 V-A + Independent	—
8 M/66 (fig. 3)	HAD, CT (old)	No	6	73	103	0.17	0.21	1:1 V-A + Irregular V-A. (Wenckebach)	—
9 M/70	HAD, PE, CWP, LS	No	7	98	151	0.19	0.13	1:1 V-A + Irregular V-A. (Wenckebach)	APS
10 F/37	PHT, (R Cath)	No	7	82	130†	0.16	0.20	1:1 V-A + Irregular V-A.	APS QRS form
11 F/68	HAD, CT (recent)	No	8	51	98 to 154	0.14	0.12	Irregular V-A + Independent	APS
12 M/49	PE, CWP, PHD, TB, MI (old), MD	Yes	6	97	136	0.21	0.16	Irregular V-A +	APS

11 F/68									
12 M/49	PE, CWP, PHD, TB, MI (old), MD	Xes	5	97	136	0.21	0.16	Irregular V-A + Independent	APS
13 F/30 (figs. 4, 5)	MS (L Cath)	No	21	115	177	0.16	0.22	Irregular V-A (Wenckebach) + Independent	APS, ventricular captures, reciprocal beats, fusion
14 M/53	MI (recent), PE, CWP	No	(>2 min.)	91	143	0.16	0.18	Irregular V-A	Ventricular captures with fusion

\*During first few beats before terminal slowing.

†During run with 1:1 V-A conduction and during first few beats of other run before terminal slowing.  
Where more than one atrial mechanism is given, this means that in the same tracing some runs of ventricular tachycardia were associated with one atrial mechanism, other runs with another.

See text for discussion of column, Evidence for Ventricular Foci.

Abbreviations: AHD—coronary arteriosclerotic heart disease, APS—atrial premature systole, AS—anxiety state, CA—coarctation of the aorta, CT—cerebral thrombosis, CWP—coal workers' pneumoconiosis, DM—diabetes mellitus, HAHD—hypertensive and coronary arteriosclerotic heart disease, HD—heart disease, LS—latent syphilis, (L Cath)—tracing during left heart catheterization, MD—muscular dystrophy, MI—myocardial infarct, MS—pulmonary heart disease, PHD—pulmonary hypertension, QRS form—maintenance of QRS form of mitral stenosis, PE—pulmonary embyssema, PHD—pulmonary embyssema, PHD—pulmonary hypertension, (R Cath)—tracing during right heart catheterization, (Surg)—ectopic beats in spite of pronounced variations in intervals between ectopic beats, (R Cath)—tracing during right heart catheterization, (Surg)—tracing during surgery, TB—pulmonary tuberculosis, V-A—ventriculo-atrial, X'P'—minimum ventriculo-atrial conduction time measured from onset of QRS in lead where it was earliest to onset of retrograde P in esophageal lead.

independent of the ventricular rhythm. There were 11 cases with runs with independent atrial rhythm, but in 4, other runs were associated with V-A conduction. V-A conduction occurred with and without heart disease and with and without the use of digitalis.

One-to-one V-A conduction is illustrated in figures 1, 2, 3, 7, and 8. Irregular V-A conduction is illustrated in figures 4 and 5. The Wenckebach phenomenon with V-A conduction is illustrated in figures 2 and 5. Ventricular tachycardia with an independent atrial rhythm is illustrated in figure 6.

Incidences in a study like this need not necessarily have general applicability in view of possible variations in patient populations and circumstances determining feasibility of studying a given arrhythmia in detail by the described technic. It must be significant, however, that almost as many cases of 1:1 V-A conduction were observed in this study as the total found in the literature to date.

#### Intermittent Ventricular Tachycardia

Since the diagnosis of ventricular tachycardia with 1:1 V-A conduction was made only if the onset, or in one case the termination, of the tachycardia was observed, it follows that the study is weighted with intermittent ventricular tachycardias. Persistent tachycardias with bizarre QRS complexes and a 1:1 P-to-QRS relation whose onset could not be observed are not included in this study because of the problem of differentiation from atrial tachycardia with aberrant conduction. In some reported cases<sup>4</sup> it is impossible to say whether the tachycardia is atrial or ventricular.

In a number of the cases of this study the duration of the runs was relatively brief. Is retrograde conduction to the atria, especially 1:1 conduction, less likely to occur in the more persistent and prolonged ventricular tachycardias? This cannot be answered by the present study, but runs of 228 beats at a rate of 186 per minute (case 1, fig. 1), 40 beats at 207 per minute (case 7),<sup>27</sup> and 23 beats at 194 per minute (case 2, fig. 7) were observed with 1:1 V-A conduction, with

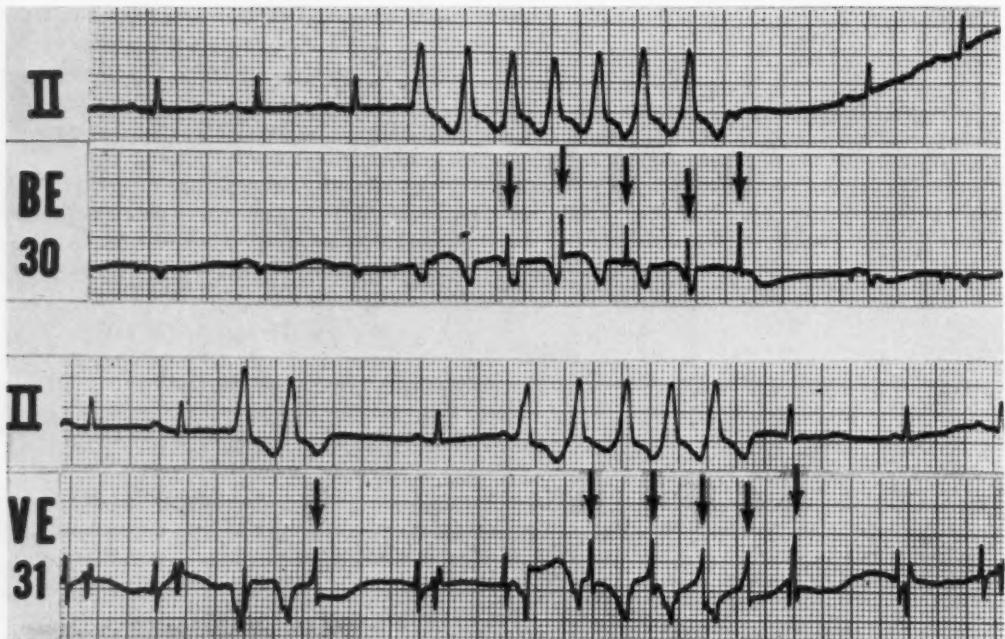


Figure 2

*Case 6. Simultaneous lead II and bipolar esophageal lead above. Simultaneous lead II and V esophageal lead below. Retrograde P waves marked by arrows. Top. Ventricular tachycardia with V-A conduction, Wenckebach phenomenon. The second, third, fifth, sixth, and seventh ectopic beats are followed by retrograde conduction. No V-A conduction after first ectopic beat because of interference with sinus beat. Wenckebach phenomenon: V-A conduction times (sec.), second ectopic beat 0.30, third 0.35, fourth blocked, fifth 0.24, sixth 0.32, seventh 0.36. Last retrograde P wave apparent in lead II. Bottom. Retrograde conduction after the second of two ventricular premature systoles near the beginning, then ventricular tachycardia with 1:1 V-A conduction starting with the second ectopic beat, and a reciprocal beat at the end. The normal QRS in lead II after the last ectopic beat is preceded by a retrograde P wave. The VE lead shows that still another retrograde P wave is superimposed on the reciprocal QRS, as if the reciprocal impulse on its way to the ventricle turned back to the atrium again. The reciprocal beat with normal QRS and thus normal conduction is suggestive evidence that the ectopic focus that initiates the tachycardia is ventricular rather than A-V nodal. The interval from the preceding ectopic beat is longer than the intervals between ectopic systoles, so that the evidence is not conclusive; the longer interval conceivably allows for recovery from a refractory phase. The pause after the reciprocal beat is ended by an A-V nodal escape beat, its QRS occurring right after a sinus P wave.*

V-A conduction times of 0.12 to 0.17 second and no progressive increase of conduction time from the beginning to the end of the run.

#### The Rate of Tachycardia and V-A Conduction

One-to-one V-A conduction occurred with ventricular rates of 91, 94, 103, 107, 130, 151, 182, 186, 194, and 207. Independent atrial

rhythm occurred with ventricular rates of 98, 120, 136, 140, 146, 150, 167, 171, 177, 207, and 273. Rate alone does not seem to be a factor determining 1:1 V-A conduction in this series, although it is possible that at ventricular rates greater than those observed in these patients, conduction might be interfered with because of rate alone. Lewis<sup>3</sup> found

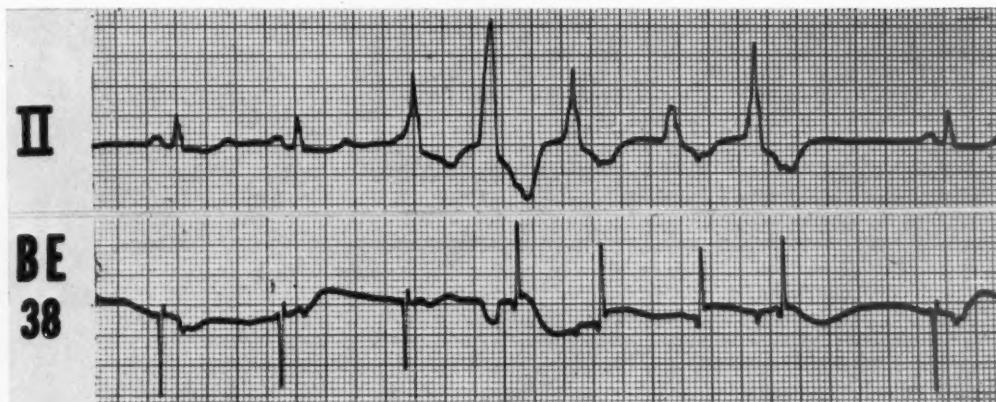


Figure 3

*Case 8. Simultaneous lead II and bipolar esophageal lead. V-A conduction after each ectopic beat except the first. No V-A conduction after first ectopic beat because of interference with sinus beat. Both sinus and retrograde P waves in the BE lead are large spiked deflections, the sinus P waves downward, the retrograde P waves upward. QRS small in BE lead. Small deflections on T waves of ectopic systoles in lead II recognizable as inverted P waves with help of the esophageal lead.*

1:1 V-A conduction frequently in dogs at rates below approximately 220; at faster rates the mechanism was usually 2:1 V-A block and rarely 4:1 V-A block.

**Differentiation from A-V Nodal Tachycardia with Aberrant Conduction**

The question may be raised whether the ectopic complexes initiating the tachycardias originate not in the ventricle but rather in the A-V node and are conducted aberrantly because of occurrence during a partially refractory phase. Evidence that most of the tachycardias with retrograde conduction to the atria in this study were of ventricular origin consists of (1) the V-A conduction times, (2) normal forward conduction with atrial premature systoles, ventricular captures by sinus beats and reciprocal beats, (3) fusion between ectopic and sinus beats and fusion between ectopic and reciprocal beats, and (4) persistence of the bizarre form of the ectopic QRS with wide variations in the intervals between ectopic complexes.

**V-A Conduction Times**

The V-A conduction times were 0.12 to 0.52 second. Briefer intervals between QRS and P such as might be expected with A-V

nodal rhythm did not occur. With the technic used it is possible to recognize P occurring simultaneously with QRS in A-V nodal rhythm and to measure small QRS-to-P intervals in such rhythm. Tachycardias in which the onset was with a P wave were not included in this study, so that so-called upper A-V nodal tachycardias are excluded.

In the five cases in which 1:1 V-A conduction alone occurred, the V-A conduction times were 0.12 to 0.20 second. In the five cases in which runs of 1:1 V-A conduction were associated with other runs with different atrial mechanisms, the V-A conduction times were 0.13 to 0.30 second. In the four cases with irregular V-A conduction and no runs with 1:1 conduction, the V-A conduction times were 0.12 to 0.52 second.

In 10 instances the V-A conduction times were about the same as P-R or longer (table 1), in four instances shorter (cases 2, 9, 11, and 12). Since the ventricular premature systole may occur during the refractory period produced by the previous systole, it is expected that the V-A conduction time will sometimes be longer than the A-V time. That the V-A time is often equal to or shorter

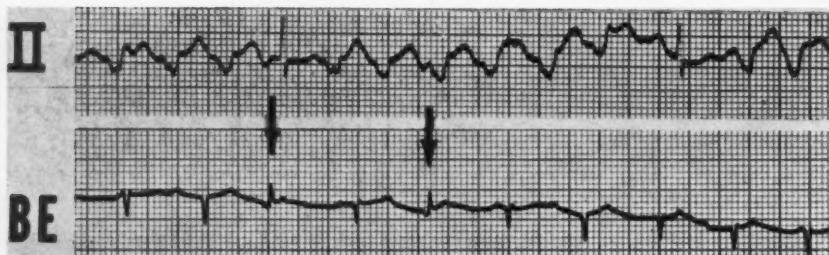


Figure 4

*Case 13. Tracing during left heart catheterization. Simultaneous lead II and bipolar esophageal lead. Ventricular tachycardia with irregular retrograde conduction, sinus beat, and reciprocal beat. In the BE lead the QRS complexes are small, rounded, upward deflections, the sinus P waves are spiked, downward deflections and the retrograde P waves spiked, upward deflections (arrows). There are two normal QRS complexes. The normal QRS toward the end of the tracing follows a sinus P. An upright  $P_2$  is visible preceding this QRS simultaneous with the spiked downward deflection in the BE lead. The normal QRS toward the beginning of the tracing follows a retrograde P wave—upward spike in the BE lead and inverted P in lead II—and is interpreted as a reciprocal beat. The occurrence of a reciprocal beat with normal QRS complex at an interval after the preceding ectopic beat similar to the intervals between ectopic beats is evidence that the ectopic focus is ventricular rather than A-V nodal.*

than A-V time requires comment, since it has been stated that V-A time in man is regularly longer<sup>34, 35</sup> and that there may normally be unidirectional block in the A-V node<sup>1</sup> so that V-A conduction is blocked or delayed compared to A-V conduction.

Studies with esophageal leads<sup>27, 32, 36</sup> show that V-A conduction occurs commonly, and while V-A intervals longer than A-V occur as expected, V-A intervals equal to or briefer than A-V occur also.<sup>27</sup> In figure 7 there seems little doubt that the tachycardia originates from a ventricular focus (see below), and yet P-R is 0.15 second and the V-A conduction time is 0.12 second. There are a number of reasons why a V-A time equal to or shorter than P-R in the same tracing cannot be used as evidence against a ventricular origin of the ectopic focus. First, the experimental evidence was previously reviewed;<sup>27</sup> the results varied, but in some studies V-A times were shorter than A-V times. Recent studies of conduction velocity in individual myocardial fibers<sup>37</sup> show that conduction through the A-V node is about as rapid in one direction as in the other. There may possibly be a site of delay in retrograde conduction dur-

ing the refractory period at the junction of the Purkinje fibers and myocardial fibers,<sup>37</sup> but even here retrograde conduction during recovery is as rapid as in the forward direction. Second, the times as measured in the clinical electrocardiogram are a crude index of conduction velocity. There may be limitations in measurement; the onset of the ectopic ventricular systole may not be recorded in the leads used, or it may be unrecognizable, being superimposed on the preceding T wave. The fiber distance traveled in V-A conduction is not the same as that in A-V conduction, the exact paths not being known, and it could conceivably be shorter. For example, from a ventricular focus near the A-V node the electrocardiographically recorded V-A time is occupied by conduction from focus-to-AV node-to first part of atrium activated, a fiber distance which could conceivably be shorter than that from atrium near S-A node-to A-V node-to first part of ventricle activated, conduction along which is represented by P-R. Third, V-A conduction could possibly occur by a different pathway with faster conductivity. There is some clinical<sup>38</sup> and experimental<sup>39, 40</sup> evidence for multiple

Table 2

*Comparison of Ectopic Systoles Initiating Tachycardia and Atrial Premature Systoles in the Same Tracing*

Patient number	Atrial premature systole followed by normal QRS		Ectopic systole initiating tachycardia	
	Duration of preceding cardiac cycle (sinus) (sec.)	Coupling, preceding QRS to QRS of atrial premature systole (sec.)	Duration of preceding cardiac cycle (sinus) (sec.)	Coupling, preceding QRS to QRS initiating tachycardia (sec.)
1:1 V-A conduction				
1	0.68	0.41	0.68	0.39
2	0.56	0.37	0.56	0.44
	0.58	0.39	0.58	0.44
5	1.12	0.63	1.12	0.73
	1.24	0.64	1.24	0.90
9	0.58	0.43	0.61	0.43
	0.60	0.38	0.60	0.46
10	0.72	0.44	0.73	0.43
	0.73	0.46	0.73	0.55
Irregular V-A conduction				
11	1.15	0.72	1.14	0.72
12	0.63	0.58	0.63	0.49
			0.65	0.53
13	0.55	0.36	0.54	0.48
	0.66	0.44	0.65	0.43

pathways of conduction. Fourth, retrograde conduction could possibly occur during a supernormal phase of the cardiac cycle. While a supernormal phase in the normal human heart has not been demonstrated, it has been observed in disease<sup>41, 42</sup> and in experimental animals.<sup>37, 43</sup>

#### *Atrial Premature Systoles*

Convincing evidence of ventricular origin of the ectopic beat initiating the tachycardia is the occurrence, in the same tracing under similar conditions, of forward conduction from above the bifurcation of the bundle of His, giving rise to normal QRS complexes. (By normal QRS is hereafter meant a QRS like one of sinus origin, although in two of the cases the QRS of sinus origin showed intraventricular block [fig. 8].) This was observed with atrial premature systoles in five of the cases with 1:1 V-A conduction (cases 1, 2, 5, 9, and 10) and in three of the cases with irregular V-A conduction (cases 11-13). The availability of atrial premature systoles for comparison was in part

fortuitous, but also in part related to the long periods of recorded observation of some of the patients. For example, two atrial premature systoles occurred in 27 minutes of recorded tracings in case 1. During cardiac catheterization and cardiac surgery the occurrence of both atrial and ventricular premature systoles is usual.

Of the conditions that may influence refractoriness of myocardium and therefore aberrant conduction, there are two that can be measured in the electrocardiogram: (1) the duration of the cardiac cycle preceding the ectopic systole and (2) the coupling interval or the interval between the ectopic systole and the preceding ventricular systole. Aberrant conduction should occur more readily with longer preceding cardiac cycles and shorter coupling intervals.<sup>44</sup> In table 2 some ectopic systoles initiating the tachycardias were selected for comparison with some atrial premature systoles in the same tracing giving rise to normal QRS complexes. The evidence is against an A-V nodal focus with aberrant

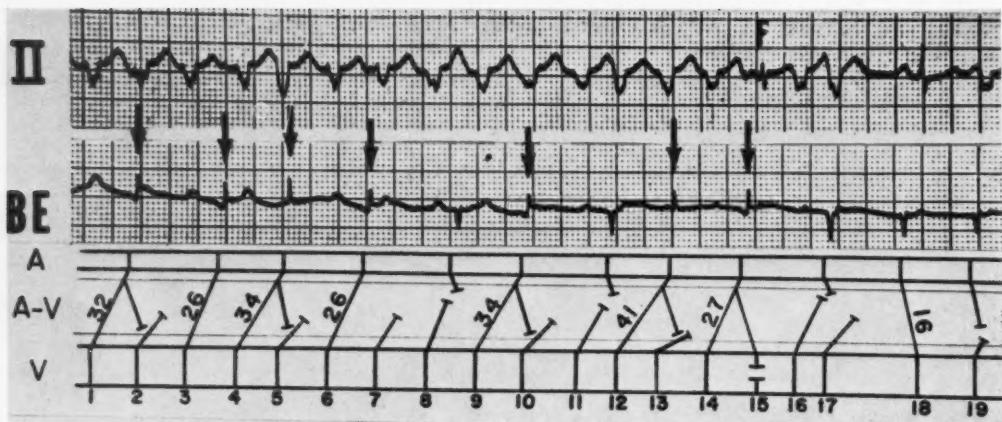


Figure 5

*Case 13. Tracing during left heart catheterization. Ventricular tachycardia with irregular V-A conduction, Wenckebach phenomenon, ventricular fusion between reciprocal and ectopic beats. In the BE lead the QRS complexes are small, rounded, upward deflections, the sinus P waves are spiked, downward deflections and the retrograde P waves are spiked, upward deflections (arrows). The next to last QRS is normal and follows a sinus P wave. At F is a complex which seems intermediate between the normal QRS and the ectopic QRS. F follows a retrograde P—upward spike in the BE lead and suggestion of an inverted  $P_2$ —and is interpreted as fusion of a reciprocal beat and an ectopic beat. The occurrence of F is evidence that the ectopic focus is ventricular. Diagram—A = atrium; A-V = A-V node; V = ventricle. Horizontal lines within A-V node designate sites of junction between retrograde and reciprocal paths. Ventricular systoles numbered in sequence below the diagram. Numbers on the diagonal lines are conduction times in hundredths of a second. The diagram shows (1) ectopic beats conducted to the atria, (2) a sinus beat (18th), (3) ventricular fusion of a reciprocal and an ectopic beat (15th), (4) failure of reciprocal impulses from some ectopic beats to reach the ventricle because retrograde conduction from the immediately following ectopic beat produces refractoriness of the part of the conduction path common to retrograde and reciprocal impulses, (5) interference between retrograde and sinus impulses preventing the appearance of retrograde P waves after some ectopic beats. V-A conduction after the 3rd, 4th, 5th, and 6th ectopic beats according to the Wenckebach phenomenon; V-A conduction times (sec.), 3rd beat 0.26, 4th 0.34, 5th blocked, 6th 0.26. Reciprocal impulses are not shown with incompletely retrograde impulses whose V-A time is not known. Neither are they shown with V-A conduction times less than 0.27 sec. (3rd, 6th ectopic beats) since 0.2 sec. is the minimum known V-A time associated with reciprocal beating; the data are inadequate, however, to determine the true minimum V-A time permitting reciprocal conduction, so that reciprocal impulses may possibly have occurred after the 3rd and 6th ectopic beats.*

conduction, except that this possibility is not ruled out in case 12 in which the coupling of the bizarre QRS is a little shorter than that of the QRS of the atrial premature systole.

#### *Ventricular Captures, Reciprocal Beats, Fusion Beats*

Forward conduction with normal QRS occurred also with ventricular captures from

sinus beats during the tachycardia and fusion of sinus and ectopic beats<sup>18, 23, 45</sup> (cases 13 and 14), and reciprocal beats (cases 6 and 13) and fusion of reciprocal and ectopic beats<sup>18, 19, 46</sup> (case 13). Reciprocal beats with normal QRS are illustrated in figures 2 and 4, and a ventricular fusion of reciprocal and ectopic beats is illustrated in figure 5. The

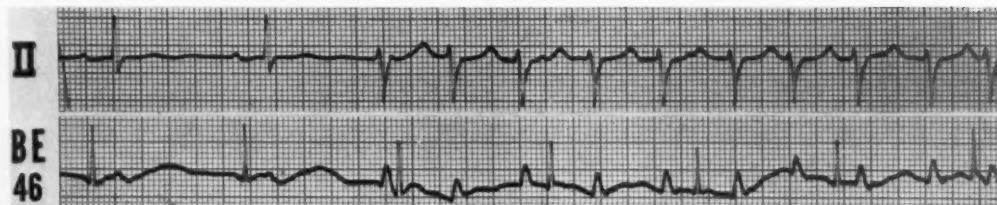


Figure 6

From one of the cases of ventricular tachycardia with independent atrial rhythm. Simultaneous lead II and bipolar esophageal lead. In the BE lead the P waves are large, upward spikes, easily followed through the run of tachycardia maintaining the sinus rhythm with slight arrhythmia.

interval between the reciprocal beat and the preceding ectopic beat in figure 4 is close to the intervals between ectopic beats, suggesting a ventricular focus of the ectopic beats. The fusion beat in figure 5 practically excludes the possibility that the ectopic beats arise in an A-V nodal focus. In figure 2 the occurrence of the reciprocal beat with normal QRS is highly suggestive that the ectopic focus is ventricular, but it is not conclusive. The interval between reciprocal beat and previous ectopic beat is longer than the interval between ectopic beats during the tachycardia, and recovery from a refractory period for the reciprocal beat is possible.

The diagram of figure 5 illustrates the interpretation that reciprocal impulses from some ectopic beats fail to reach the ventricle because retrograde conduction from the immediately following ectopic beat produces refractoriness of the part of the conduction path common to retrograde and reciprocal impulses. Such a mechanism was postulated by Pick and Langendorf.<sup>46</sup>

#### Persistence of Form of QRS with Varying Intervals

In two cases of 1:1 V-A conduction (cases 4 and 10) the persistence of the form of the ectopic QRS, in spite of pronounced prolongation of the interval between ventricular complexes, is evidence of ventricular rather than A-V nodal origin of the ectopic focus. In figure 8 toward the end of the illustrated run, the interval between ectopic systoles is close to that between the sinus beats. There seems no reason why an A-V nodal focus

should be conducted aberrantly at this time, nor why the form of aberration should remain identical in spite of such pronounced variations in the intervals between ectopic systoles. A ventricular focus with a uniform path of spread through the ventricles regardless of the intervals between ectopic beats seems more likely.

#### The Form of QRS

It is believed that the QRS more frequently assumes the pattern of right bundle-branch block in aberrant ventricular conduction,<sup>47</sup> although the pattern of left bundle-branch block may occur also.<sup>48</sup> There was no right bundle-branch block pattern in cases 1, 7, 8, and 14. There was a pattern possibly of atypical right bundle-branch block in cases 9 and 11. In the other patients the tachycardia was not observed in the leads necessary for the diagnosis; some patients with infrequent runs of tachycardia were observed as long as possible on lead II and esophageal leads, and the same procedure was used for observation during surgery and cardiac catheterization.

To complete the discussion of the differentiation of ventricular from A-V nodal systoles one must refer to the interesting observations of Rakita, Kennamer, Rothman, and Prinzmetal<sup>49</sup> that experimental irritation of the A-V node may produce bizarre QRS complexes. According to the authors this occurs when the A-V node is injured, only part of the fibers from the node being activated, these fibers passing far out into the ventricle without anastomosis with adjacent fibers. Whether

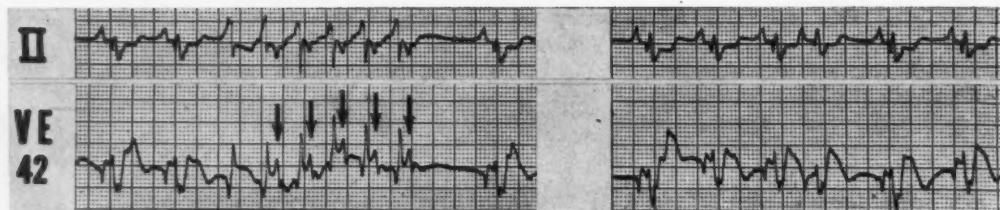


Figure 7

*Case 2. Simultaneous lead II and V esophageal lead. Comparison of preceding cardiac cycle and coupling interval of ectopic systole initiating tachycardia (left) and atrial premature systole (right) from same tracing. This illustrates the method of table 2. A-V conduction after the atrial premature systole giving rise to a QRS like the QRS after a sinus beat at intervals comparable to the ectopic systole that initiates the tachycardia is evidence that the ectopic focus is ventricular. Ventricular tachycardia with 1:1 V-A conduction starting with the second ectopic beat (left), retrograde P waves marked by arrows. No retrograde conduction after first ectopic beat because of interference with sinus beat. This tracing is exceptional in the series in that the retrograde P waves show clearly in lead II. A similar consecutive run of 23 beats was observed with no change in V-A conduction time.*

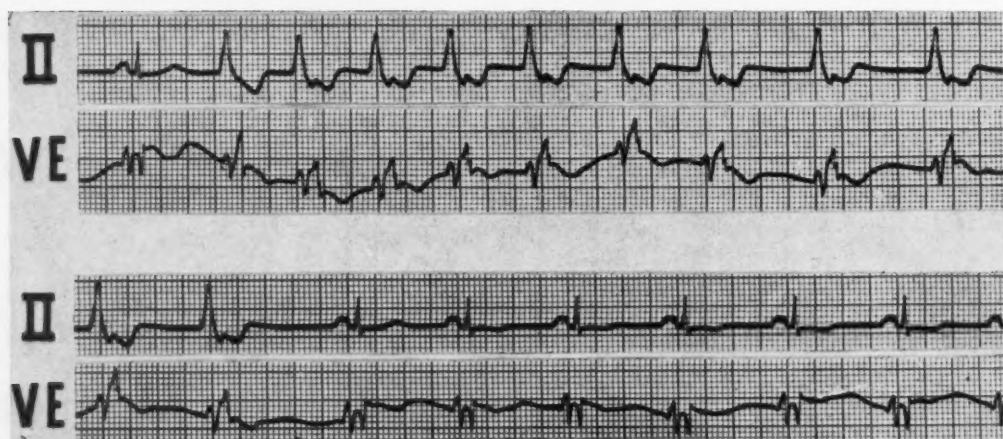


Figure 8

*Case 4. Simultaneous lead II and V esophageal lead. Lower strips continuous with upper. Ventricular tachycardia with 1:1 V-A conduction and pronounced slowing of ventricular rate. QRS form of the ectopic beats maintained even when interval between ectopic beats equals or exceeds that between sinus beats. This is evidence that the ectopic focus is ventricular, since at these longer intervals there is no reason for aberrant conduction from an A-V nodal focus. The peaks of the retrograde P waves in the VE lead are 2 to 5 mm. above the peaks of the ectopic QRS. First ectopic QRS probably superimposed on sinus P.*

this is clinically significant, and whether anything like such a mechanism could be involved in cases without clinical evidence of A-V nodal injury, one cannot say at present. If an ectopic focus in part of the A-V node could produce bizarre QRS complexes by

the suggested mechanism of Rakita et al. while an impulse from the atrium or a reciprocal route could pass through the A-V node to the ventricle to produce normal QRS complexes, then some of the evidence presented here for the ventricular origin of

the ectopic foci would not be conclusive. Nothing more can be said at present about such a possibility, which would question the origin of the common premature systoles conventionally considered ventricular.

**Differential Diagnosis of Ventricular and Supraventricular Tachycardia**

Should 1:1 V-A conduction in ventricular tachycardia occur with anything like the frequency suggested by this study, then the problem of differentiation between ventricular tachycardia and supraventricular tachycardia with aberrant conduction is more complicated than has been supposed. On the assumption that 1:1 V-A conduction in ventricular tachycardia is rare, the finding in esophageal tracings of a 1:1 relation of QRS and P has been used as evidence of supraventricular tachycardia.<sup>50</sup> It has previously been emphasized<sup>23</sup> that this is no absolute distinction, and the present study may indicate that it does not have even a probability value in differential diagnosis. The serious limitations of some of the criteria in use for the diagnosis of ventricular tachycardia have been thoroughly analyzed.<sup>23, 46, 51</sup> This study casts additional doubt on one of the classical criteria, namely, the independent atrial rhythm, which may be absent in ventricular tachycardia more frequently than has been realized.

**Summary and Conclusions**

Ventriculo-atrial (V-A) conduction in ventricular tachycardia has been recognized relatively frequently in studies with simultaneous esophageal and standard leads. Of 21 cases interpreted as ventricular tachycardia there was 1:1 V-A conduction alone in five, 1:1 V-A conduction in some runs of tachycardia with other mechanisms in other runs in five, V-A conduction with variable block in four, and an independent atrial rhythm alone in seven.

Evidence that the ectopic foci in these cases are indeed ventricular rather than A-V nodal with aberrant conduction is based on (1) V-A conduction times, (2) normal forward conduction with atrial premature sys-

toles, ventricular captures by sinus beats and reciprocal beats, (3) fusion between ectopic and sinus beats and fusion between ectopic and reciprocal beats, and (4) persistence of the bizarre form of the ectopic QRS in spite of varying intervals between ectopic beats.

The frequency of 1:1 V-A conduction in ventricular tachycardia complicates the differential diagnosis from supraventricular tachycardia with aberrant conduction.

A bipolar esophageal lead is often superior to a V esophageal lead for the study of complex arrhythmias and V-A conduction. It is more likely than a V esophageal lead to show retrograde P waves more or less opposite in direction to the sinus P waves.

**Acknowledgment**

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### Heart Failure

It is impossible thoughtfully to survey, in the light of daily experience, the field of medical work covering diseases of the heart, varied as the manifestations may be, without realising the central problem to be failure of the heart to accomplish its work in lesser or greater degree. This work consists in the propulsion of blood through the circle of vessels in adequate quantity to meet the needs of the body in the ordinary and varied circumstances of life. The very essence of cardiovascular practice is recognition and early heart failure and discrimination between different grades of failure. This simple truth is not stated here for the first time; in theory it receives occasional homage from many. It emerges into view for a fleeting moment, to retreat and lie concealed beneath a mass of technical, and by comparison trivial, detail; it does not dominate cardiae practice as it should. When a patient seeks advice and heart disease is suspected, or is known, to be present, two questions are of chief importance. Firstly, has the heart the capacity to do the work demanded of it when the body is at rest? Secondly, what is the condition of the heart's reserves? These questions can be answered, and correctly answered, in almost all cases by simple interrogations and by bedside signs; and the answers force all other considerations into the background in most cases of chronic heart disease; they are essentials to sound prognosis and treatment.—SIR THOMAS LEWIS. *Diseases of the Heart*. New York, The MacMillan Company, 1933, p. 1.

## The Reopened Ventricular Septal Defect

### A Syndrome Following Unsuccessful Closure of Interventricular Septal Defects Particularly in Association with Infundibular Stenosis

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SINCE the first open intracardiac repair of interventricular septal defects and tetralogy of Fallot,<sup>1, 2</sup> rapid progress has been made in operative techniques and in extracorporeal perfusion technology. Operative risk has steadily declined, and recent communications indicate that in ventricular septal defects uncomplicated by pulmonary hypertension the mortality rate is approximately 2 per cent.<sup>3-5</sup> In tetralogy of Fallot the mortality risk is in the vicinity of 12 to 16 per cent,<sup>6, 7</sup> but this incidence is also diminishing.<sup>5, 7</sup>

The results after successful closure of a ventricular septal defect and the correction of Fallot's tetralogy are gratifying.<sup>4, 7, 8</sup> Symptoms disappear or are markedly alleviated. The precordium becomes quiet, and the typical murmurs cease or are dramatically modified. Cyanosis disappears. Radiographically, there may be a reduction in heart size, and the plethora of the lung fields regresses. It soon became apparent, however, that the therapeutic benefits of complete closure were not always achieved because of disruption of the repair, and the technic was refined.<sup>9, 10</sup> Although the first disruptions were reported in attempted closure of uncomplicated ventricular septal defects and continue to be described in more recent reports pertaining to the treatment of this lesion,<sup>11-13</sup> it has been the experience in this laboratory that the per-

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sistence of residual defects after surgery is rare in uncomplicated ventricular septal defects but that it is particularly a problem in the repair of tetralogy of Fallot. Similar opinions have been expressed elsewhere,<sup>14, 15</sup> and we believe that this point deserves particular emphasis; for the clinical consequences of incomplete or impermanent closure of a ventricular septal defect, during a procedure in which infundibular stenosis has been successfully relieved, are appreciable and at times alarming.

In some instances the left-to-right shunt through the lungs may be huge, and both the pulmonary circulation and left ventricle are presented abruptly with unaccustomed loads. Two deaths after progressive hypotension following repair for tetralogy of Fallot have been ascribed, at least in part, to incomplete ventricular septal defect repair.<sup>7</sup> It is the purpose of this paper to describe more fully the clinical and hemodynamic consequences of unsuccessful closure of ventricular septal defect, particularly in association with infundibular stenosis, and to discuss the modifications of surgical technics that show promise of minimizing the incidence of unsuccessful closure.

#### Material and Methods

The material of the study consists of seven patients who underwent open intracardiac repair at Stanford University Hospital between March 1956 and December 1959. Six of the seven patients had ventricular septal defects complicated by infundibular stenosis or a hypertrophied crista supraventricularis. The seventh patient had an uncomplicated ventricular septal defect. All patients had extensive preoperative clinical and catheterization studies. Postoperative clinical information, cardiac roentgenograms, and electrocardiograms were reviewed. The diagnosis of persisting left-to-right shunt was made by the recognition of a distinctive clinical syndrome and was confirmed by

cardiac catheterization. In each instance a second operative closure was performed and the outcome was evaluated by careful and repeated observations.

#### Case Reports

##### Case 1

D.S. was an 11-year-old girl in whom a heart murmur was heard at age 2. In December 1957, at age 10½, she was admitted for study, having experienced subnormal growth and easily induced dyspnea. Severe exertion was not tolerated. There was no cyanosis at rest or mild exercise.

On examination there was a "pigeon-breast" deformity of the sternum. There was a systolic heave at the left sternal border, and a palpable thrill was present low along the sternum. A grade 4/4 full systolic murmur was heard in this area. It was well transmitted over the precordium and back. There was a diastolic rumble at the apex. The second sound at the pulmonic area was not widely split and not accentuated.

Roentgenograms revealed biventricular enlargement, a prominent main pulmonary artery, hypervascular lung fields, a right-sided aortic arch, and an anomalous left subclavian artery. The electrocardiogram was compatible with right ventricular hypertrophy. The clinical and laboratory data were consistent with the catheterization findings elsewhere (August 1954) of an interventricular septal defect. There was mild elevation of the pulmonary artery pressure, and pulmonary stenosis was absent.

The patient underwent cardiopulmonary bypass surgery on March 6, 1958. After potassium arrest and right ventriculotomy, an interventricular septal defect measuring 2.5 cm. in diameter was repaired with a continuous suture line reinforced by interrupted sutures. The defect had a fibrous wall on the right ventricular side of the aortic valve, and a fibrous approximation was possible, but access was difficult because of a hypertrophied crista supraventricularis. During the next 48 hours there were persisting hypotension, pallor, and restlessness, in spite of blood replacement and hydrocortisone. On March 8, cardiac tamponade was diagnosed. The sternotomy incision was reopened, the pericardium evacuated of 500 ml. of sanguineous fluid, and the blood loss was replaced. The vital signs then stabilized and the patient's condition improved, but she continued to be dyspneic at rest. The precordium remained active. A left sternal border thrill and a loud systolic murmur were present low along the sternum. There was a left ventricular heave but no third sound or rumble. The second sound was widely split, coinciding with the appearance of a pattern of right bundle-branch block in the

electrocardiogram. The liver was 3 or 4 cm. down. On recatheterization, it was confirmed that a left-to-right shunt was still present, and the values for pulmonary blood flow and pressures were practically identical to those of the original study 4 years before. On April 10, 4½ weeks after the original procedure, repair was attempted once more. The upper two thirds of the repair was intact, but the lower one third adjacent to the tricuspid valve had come loose. The hypertrophied bar of crista supraventricularis was removed for better access and an Ivalon sponge was sutured to the repair with purse string sutures, additionally reinforced with interrupted sutures. The repair appeared firm and the postoperative course was uneventful. The precordium was now quiet and the intense murmur and thrill were gone. The patient was discharged and has been followed without evidence of further reappearance of the shunt.

##### Case 2

J.L., a 13-year-old boy with a heart murmur heard at birth, was first studied in October 1956. He had experienced normal growth and development, and was able to participate in competitive sports without difficulty. There was no history of cyanosis. On examination he was a husky lad with an obvious left sternal border thrill and a grade 4/4 pansystolic murmur heard all over the precordium but maximal at the pulmonic area. Pulmonic closure could not be heard but was recorded as a delayed, faint sound on the phonocardiogram. There was prominence of the left hemithorax, which was hyperactive. Radiographically, the left ventricle was not enlarged but there was some prominence of the right ventricular silhouette as well as of the central pulmonary vessels. There was suggestive evidence of increased pulmonary blood flow. The electrocardiogram was indicative of right ventricular hypertrophy. Cardiac catheterization established the diagnosis of infundibular stenosis, and an interventricular septal defect with left-to-right shunt (table 1).

He underwent open heart surgery on March 25, 1958. The right ventricle was incised, and a large oval defect measuring 2.25 cm. in transverse diameter was located in the muscular portion of the septum just above the tricuspid valve and beneath the orifice of the aorta. The lowermost corner of the defect was first approximated with interrupted sutures of 3-0 silk, after which an Ivalon prosthesis was interposed and secured by three mattress sutures passing through the tissues on each side. About 9 Gm. of muscle were removed from the outflow tract, and additional muscle masses were excised below the area of stenosis.

On the third postoperative day, sinus tachycardia was present and a pansystolic murmur

Table 1  
Cardiac Catheterization in Case 2

Preoperative— October 16, 1956	Postoperative —April 24, 1958	
O <sub>2</sub> ml./100 ml. blood		
SVC	14.6	9.5
IVC	15.1	
RA	14.5, 15.2	9.9, 9.8
RV	16.5, 16.6	15.5, 15.7
PA	16.9, 17.2	15.3
BA	19.4 (91%)	15.9 (90%)
Capacity	21.2	17.6
Pulmonary-systemic flow ratio	1.9/1	6.0/1
Pressures, mm. Hg		
RA	2, mean	13, mean
RV infund.	19/6	
RV	110/6	55/11
PA	23/10	56/27
PA mean	18	40
PA wedge, mean		24
BA	142/87	131/65
Estimated O <sub>2</sub> consumption ml./min.	230	288
Pulmonary A-V difference ml./100 ml.	1.9	1.0*
Pulmonary blood flow, L./min.	12.0	29.0†
Pulmonary vascular resistance units	1.1	0.5†

\*1.0 ml./100 ml. for the pulmonary A-V difference is arbitrarily assumed, since the true oxygen content of pulmonary artery blood probably cannot be determined under these circumstances.

†These values for pulmonary blood flow and vascular resistance were derived from the assumed pulmonary A-V difference of 1.0 ml./100 ml.

of maximal intensity could be heard along the lower left sternal border. During the following 2 weeks he appeared to be doing well except for a persisting tachycardia. On April 10 he was noted to be dyspneic at rest. The pulse rate was 96, and the liver was large and tender. A thrill and an intense systolic murmur were present along the lower left sternal border, and a distinct third sound was now present at the apex. X-rays showed an increase of heart size, and the lung fields were diffusely engorged, suggesting pulmonary edema. On April 16 he was pale and breathless. The precordium was hyperdynamic, and the pulse was small and rapid. Rales were present at the right base. That evening he coughed up 40 ml. of frothy red sputum and was tachypneic and anxious. The neck veins were full, and systolic pulsations were present. Moist rales were again heard. He was treated for congestive failure with

digoxin, diuretics, oxygen, and hypnotics. By April 21 his symptoms had improved and there was radiographic clearing of the pulmonary edema. He was recatheterized at this time. The pulmonary stenosis had been completely relieved but there was a 29-liter pulmonary blood flow indicating that the interventricular septal defect was again patent. A second repair was undertaken on May 6. The medial margin of the repair had separated. The Ivalon prosthesis had remained adherent on the right side, but was free on the left. The defect now had a firm fibrous rim that could be approximated with continuous sutures and closed with interrupted stiches. The closure line was then covered by compressed Ivalon fastened down by continuous over-and-over sutures. On the third postoperative day he became progressively dyspneic and the pulse was rapid. The sternotomy was reopened and a liter of blood removed from the thorax. The pericardium was evacuated of clot and 200 ml. of blood. There was immediate improvement and the postoperative course was subsequently uneventful. The precordium was now quiet and there was no evidence of congestive failure. In follow-up examinations he has maintained his improvement and now feels entirely well.

#### Case 3

F.H., a 37-year-old woman, had a heart murmur from birth as well as an episode of polyarthritis at age 12. She had always been on activity restriction. On her initial admission she complained of exertional dyspnea. There had been no cyanosis.

On examination there was a right ventricular lift and a left ventricular heave outside the midclavicular line. A thrill and a grade 4/4 full systolic murmur were present at the lower left sternal border. At the apex a distinct third sound was present. The second sound was widely split to auscultation and on the phonocardiogram. Cardiac views showed biventricular enlargement, large main pulmonary vessels, and hypervascular peripheral lung fields. The electrocardiogram was indicative of right bundle-branch block. Cardiac catheterization, January 10, 1958, demonstrated the presence of infundibular stenosis with a left-to-right shunt through an interventricular septal defect (table 2).

The patient underwent operative repair on March 11, 1958. After potassium arrest on cardiopulmonary bypass, a ventriculotomy was made and 6 Gm. of muscle were removed from the infundibulum. An interventricular septal defect measuring 15 mm. in transverse diameter was located adjacent to the aortic valve. The defect had a fibrous edge. An Ivalon prosthesis was sown into place with interrupted 3-0 silk sutures; this was further reinforced by sewing a redundant

Table 2

Cardiac Catheterization in Case 3

Preoperative— January 10, 1958	Postoperative —Jun. 6, 1958	
O <sub>2</sub> ml./100 ml. blood		
SVC	10.1	8.3
IVC	8.1	
RA	8.9, 9.4	9.0, 9.1
RV	11.7, 11.8, 12.9	11.6, 11.8, 12.0
PA	12.6, 12.9	11.1, 11.2
BA	14.0 (92%)	13.7 (93%)
Capacity	15.3	14.8
Pulmonary-systemic flow ratio	3.4/1	3.0/1
Pressures, mm. Hg		
RA	5, mean	5, mean
RV infund.	30/2	
RV	117/4	36/6
PA	29/12	33/12
PA mean	22	23
BA	100/49	
Estimated O <sub>2</sub> consumption ml./min.	197	215
Pulmonary A-V difference ml./min.	1.4	2.5
Pulmonary blood flow, L./min.	14.0	8.6
Pulmonary vascular resistance units	1.2	2.1

leaf of the tricuspid valve over the repair. On March 13 there was an abrupt onset of tachycardia, rate 200, accompanied by pallor, sweating, and hypotension. Pericardial tamponade was feared, and the patient was re-explored by opening the sternotomy incision. There was no free blood but the right upper lobe was collapsed and the pulmonary artery was distended. Two-hundred and fifty milliliters of blood were removed from this vessel, and the patient was rapidly digitalized. Her general condition improved and she was now in atrial fibrillation. Definite orthopnea was present. On March 19 her jugular veins were tense, the liver was four fingerbreadths below the costal margin; a loud systolic murmur along the left sternal border and right basilar rales were noted. Her failure was controlled with chlorthiazide and further digitalization. On March 26 sinus rhythm was restored with quinidine. By April 4, at the time of discharge, she felt improved, but there were venous systolic pulsations and a 4/4 pansystolic murmur along the left sternal border followed in diastole by a blowing murmur of pulmonary insufficiency.

She was re-examined on June 2, 1958. In the interim she had experienced breathlessness on slight exertion and angina on hill climbing. The

Table 3

Cardiac Catheterization in Case 4

Preoperative— September 26, 1957	Postoperative —April 20, 1959	
O <sub>2</sub> ml./100 ml. blood		
SVC	15.2	9.5
IVC		10.9
RA	15.1	9.8, 10.3
RV	16.4, 16.9	12.1, 13.0
PA	16.1	12.0, 11.8
BA	19.5	14 (92%)
Capacity	19.8	15.2
Pulmonary-systemic flow ratio	1.3/1	2.0/1
Pressures, mm. Hg		
RA	3, mean	10, mean
RV infund.	27/4	
RV	79/3	79/10
PA	13/7	30/16
PA mean	10	23
BA	75/50	111/65
Estimated O <sub>2</sub> consumption ml./min.	180	197
Pulmonary A-V difference ml./100 ml.	3.4	2.1
Pulmonary blood flow, L./min.	5.3	9.4
Pulmonary vascular resistance units	1.0	1.9

veins were flat at this time, but there were distinct right and left ventricular heaves and a maximal intensity pansystolic murmur and thrill low along the left sternal border. The second sound was loud, and a soft diastolic murmur of pulmonary insufficiency was heard. The liver was not enlarged. The electrocardiogram continued to show right bundle-branch block. Cardiac catheterization indicated that the infundibular stenosis had been removed successfully but that a large left-to-right shunt persisted. On September 17, reclosure was performed. The inferior portion of the defect was again patent just above the attachment of the tricuspid leaflet. There was dense surrounding scar tissue and the Ivalon could not be identified. The defect could be firmly closed by interrupted sutures. The patient's postoperative course was uneventful, and her precordium was quiet. In follow-up examination she has felt much improved, and it is apparent that her defect has remained closed.

#### Case 4

RiJo is a 15-year-old boy in whom a heart murmur was heard at birth. His growth and development were somewhat subnormal and he had experienced a gradual decrease in exercise tolerance. On examination there was a marked

thrill along the left sternal border accompanied by a grade 4/4 systolic murmur, which was well transmitted to the apex, aortic area, neck, and axillae. The second sound components were broadly separated. The electrocardiogram pattern was indicative of incomplete right bundle-branch block. X-rays were suggestive of right ventricular enlargement, and the pulmonary vascular markings were increased, suggesting a left-to-right shunt. Cardiac catheterization done elsewhere 2 years before was indicative of infundibular stenosis with a small left-to-right shunt (table 3).

Cardiopulmonary bypass without cardiac arrest was performed on March 31, 1959. A ventriculotomy was made, and 4 Gm. of muscle were removed from the infundibulum. An interventricular septal defect was located just proximal to the aortic valve. With ventricular systole the defect appeared as a split with a linear measurement of 3 to 4 mm. A woven Teflon graft was sewn over the defect with mattress sutures, care being taken to include a good quantity of surrounding muscle in the sutures. Access was difficult because of the large crista supraventricularis. There was slight oozing across the porous fabric of the graft, which appeared to be in good position.

The immediate postoperative course was uneventful, but on April 5 a thrill and loud systolic murmur were again present. There were a persistent tachycardia, rales at the right base, and a four-fingerbreadth tender liver. On x-ray examination right pleural effusion was identified. Recatheterization on April 21 indicated a persisting gradient across the pulmonary valve but the pulmonary artery pressure had become normal. Evidence of a left-to-right interventricular shunt was still present.

On April 29 reclosure was performed. The partially detached Teflon graft was reapproximated through an atriotomy incision, and then the ventriculotomy was reopened for the purpose of reinforcing the closure with a Teflon sponge. On the first postoperative day after signs of tamponade had appeared, the sternotomy had to be reopened and clots removed from the pericardium. The subsequent course was marked by recurrent right pleural effusion and fever. The patient improved after repeated aspiration of sterile fluid, and there was gradual defervescence. He then became asymptomatic and was discharged on May 25. On follow-up examination, he has remained well; there has been no evidence of further disruption of his interventricular septal defect repair.

#### Case 5

B.L.M., a 17-year-old boy, was admitted for open-heart surgery on October 8, 1958. A murmur had been detected at 3 months of age. The patient

had been a frail child who ate poorly and gained weight slowly. There had been intermittent cyanosis but the time of onset was not known. Frequent pneumonia and episodes of subacute bacterial endocarditis had made numerous hospitalizations necessary. Cardiac catheterization in September 1952 indicated the presence of a bidirectional shunt at ventricular level and pulmonary stenosis.

On examination the patient was small and thin. Moderate cyanosis and clubbing were present. There was slight, left precordial bulge, and the apex impulse was within the midecostal line. At the third left intercostal space there was a grade II blowing systolic murmur, and the second sound could not be heard in the pulmonary area. A right ventricular hypertrophy pattern was present in the electrocardiogram. Cardiac views demonstrated slight right ventricular prominence and slightly diminished pulmonary vascularity. A selective angiogram confirmed the presence of an interventricular septal defect with pulmonary stenosis and a right-to-left shunt. The packed cell volume was 64; hemoglobin, 19.1 Gm.

On October 15 at open-heart surgery a conical valvular stenosis with a 4-mm. orifice was found. This was opened in both directions, producing a bicuspid valve. Five grams of infundibular muscle were removed, and an interventricular septal defect measuring 14 mm. in diameter was identified just above the insertion of the tricuspid valve. The defect was sutured mattress-fashion, the edges being apposed with four sutures; this was reinforced posteriorly by a flap of redundant tricuspid valve. An Ivalon prosthesis was then anchored over the linear repair with continuous over-and-over sutures.

The first postoperative week was marked by dyspnea, tachypnea, and mild cyanosis. There were some neck vein distention, a 3-cm. hepatomegaly, and basal rales. The electrocardiogram now showed a pattern of right bundle-branch block and x-rays demonstrated congested lung fields and free fluid in the bases. The venous pressure was 26 cm. of saline, and the circulation time was 33 seconds. In spite of digitalization and other measures, heart failure persisted. There was an irregular fever, which reached 39.4 C. in the third postoperative week. Blood cultures were positive for *Staphylococcus albus*, coagulase positive and *Bacillus subtilis*, but these were thought to be contaminants. The clinical situation was grave. Examination now revealed pulsating neck veins and a pansystolic murmur of maximal intensity along the left sternal border, over the precordium and back. The spleen and liver were enlarged to the umbilicus. Repeat catheterization on December 9 showed persistence of a left-to-

right shunt at ventricular level and pulmonary stenosis.

A second open-heart procedure was performed on December 23, and a hole was identified in the lowermost portion of the repair where the Ivalon had become detached laterally and posteriorly from the tricuspid corner. The defect was again repaired with mattress sutures, the Ivalon being repositioned. The closure was further reinforced by a suture placed deep in the tricuspid valve and encompassing the defect. More muscle was removed from the infundibular region. Sections from some necrotic muscle at the edge of the defect contained clumps of gram-positive cocci and endocardium from the region of the sutures showed acute and chronic endocarditis. There was now less respiratory distress, the venous engorgement was diminished, the liver became smaller, and the precordium was quieter, a much softer systolic murmur now being present along the left sternal border. But the temperature continued to rise to 39.9 C., *Staph. aureus* appeared in blood cultures, and the right shoulder became swollen and tender. On Jan. 4, 1959, the right shoulder joint was opened and drained and osteomyelitis was found in the humerus. X-rays also revealed mottled lucencies in the acromial process. The patient received penicillin, chloramphenicol, novobiocin and streptomycin, but fever to 40.5 C. continued and the blood cultures remained positive for *Staph. aureus*. On January 31 the doses of chloramphenicol and novobiocin were increased to 2.0 Gm., and vancomycin was started. The patient then became afebrile. The blood cultures were sterile until February 19, 3 days after the discontinuation of all antibiotic treatment. The previous regimen of antibiotics was begun once more, and the temperature became normal again on March 8. Bacitracin was administered for 4 days, but was discontinued because of a blood urea of 108 mg. In addition, a precordial thrill was again palpated, loud systolic and diastolic murmurs were heard along the left sternal border, and the liver was larger. On April 11 chills and a temperature of 39.5 C. were accompanied by pain in the right ankle, and the blood cultures again contained *Staph. aureus*. Erythromycin, 8 Gm., and ristocetin 4 Gm. were started. The patient became afebrile again and the cultures were sterile; but the general condition was poor. Hypotension and oliguria were present, and the blood pressure was maintained with the help of norepinephrine. On April 21 pulsating neck veins, large liver, right ventricular heave, and a maximal left sternal border systolic murmur indicated a reopened interventricular septal defect and tricuspid insufficiency. This was confirmed by catheterization on May 11. The following day an attempt was made

to close the defect again. This was accomplished but postoperatively circulatory failure was intractable, and the patient died within 24 hours.

At autopsy the heart weighed 520 Gm. The right ventricle was hypertrophied and dilated. The pulmonic valve was bicuspid, and the cusps were deformed and folded back upon themselves. The recent interventricular septal defect repair was intact. On the left ventricular side of the defect a small vegetation was identified. Infarcts were present in the spleen and left kidney, and thrombi were present in the iliac veins and moderate-sized pulmonary arteries. There were edema of the lungs, congestion of other viscera, and ascites.

#### Case 6

RoJa, a 26-year-old man, was first admitted on March 3, 1959. In spite of a history of heart murmur from birth, there were no symptoms or limitation of activity, no cyanosis, or disability. On examination there were a slight right ventricular heave and a thrill at the lower left sternal border. There was a maximal intensity, full systolic murmur in this position. The second sound was heard better at the aortic area, and no splitting could be detected. Cardiac catheterization indicated the presence of a left-to-right shunt at the ventricular level and infundibular stenosis. The electrocardiogram pattern was right ventricular hypertrophy but there was only questionable chamber enlargement radiographically. The pulmonary artery was dilated but the vascularity of the lung fields was normal.

Open-heart surgery was performed on May 28. There was a 2.0-cm. defect in the membranous septum and both infundibular and valvular stenosis were present. The defect was closed with six ventral sutures. The closure was reinforced by suturing Ivalon sponge over the defect with interrupted mattress sutures. Pulmonary valvotomy was done, and infundibular muscle was excised. An Ivalon prosthesis was used to enlarge the outflow tract; this was reinforced with pericardium.

A loud systolic murmur persisted at the left sternal border, and a diastolic flow murmur appeared at the apex. The precordium was now hyperdynamic, and there were jugular pulsations. Edema appeared in the sacrum and lower extremities. In spite of digitalization and other measures to control congestive failure, the patient was dyspneic, uncomfortable, and had persisting tachycardia. Radiographically, the heart was now much enlarged. The clinical picture suggested reopening of the ventricular septal defect with a large left-to-right flow, tricuspid insufficiency, and congestive heart failure. On June 15, during catheterization, ventricular fibrillation occurred abruptly, and resuscitation was effected by cardiac massage

and electrical defibrillation. During the next 24 hours there were persisting tachycardia and hypotension, and 3,750 ml. of blood were lost through the drainage tubes in the chest. The chest was reopened, and an additional 1,500 ml. of blood were evacuated, after which a bleeding intercostal artery was identified and ligated. The patient's condition then improved, but he was febrile and a heavy growth of *Aerobacter aerogenes* was present in the fluid from the chest tubes. On examination, the precordium was heaving, and jugular and hepatic pulsations were marked. The systolic murmur with maximal intensity at the left sternal border was followed by a loud diastolic rumble, suggesting a tricuspid flow murmur. The systolic murmur could easily be heard over the liver. There were ascites and pronounced edema. Fever continued in spite of multiple antibiotic therapy, and *Aerobacter* was cultured routinely from persistently draining chest tubes. Roentgenograms indicated the persistence of an extensive empyema cavity. An open drainage was done on July 27. The patient subsequently became afebrile and was discharged for interim nursing care. He was readmitted in October, at which time physical findings were essentially unchanged, and a second attempt was made to close the defect. Portions of the previously placed Ivalon were still in place but the defect was widely open. Closure was effected with five mattress sutures, and a muscle flap from the anterior portion of the septum was used for reinforcement. Several sutures were taken in the patulous tricuspid ring. Postoperatively there was profuse drainage of blood, and the right hemithorax was repeatedly evacuated. A total of 6 liters of blood was withdrawn or drained. Oliguria and hypotensive, in spite of vigorous replacement therapy, the patient died on the fourth postoperative day.

At autopsy, the heart weighed 650 Gm.; there was notable enlargement of the right ventricle. The pulmonic valve was normal but just distal to the valve, there was a fibrous ring. The circumference was 3.5 cm. The aorta arose anteriorly to and to the right of the right ventricular outflow tract. The Ivalon gusset in the outflow tract was intact. The recent interventricular septal defect closure was secure. At the apex the myocardium was greatly thinned and replaced by fibrous tissue. There were small mural thrombi attached to the endocardium of both ventricles, and an embolus was present in the left coronary artery. The lungs were edematous, hemorrhagic, and atelectatic, and generalized visceral congestion was present.

#### Case 7

M.M., was an 8½-year-old girl with a congenital heart murmur. A diagnosis of interventricular septal defect had been established by cardiac

catheterization at the age of 10 months. No significant gradient was present across the pulmonary valve. There was no cyanosis, but exertional dyspnea was present, the child did not gain weight normally, and frequent respiratory infections occurred. For 3 years prior to admission the patient had been on a low-salt diet and digitalis because of "pulmonary congestion." On examination the veins were flat, and there was no cyanosis. A right ventricular heave was present, and there was a thrill and a loud pansystolic murmur at the third left interspace along the sternal edge. The second sound was loud. On March 20, 1956, at open-heart surgery, a 15-mm. interventricular septal defect was located in the membranous septum; it was sutured with interrupted silk. Postoperatively a loud systolic murmur persisted; this could also be heard in the neck. The second sound was accentuated in the pulmonic area. Radiographically, the heart was larger than preoperatively, and there was venous engorgement. The patient was seen again in December 1957, at which time she was thought to be less dyspneic. The precordium was active, however, and a maximal intensity, left sternal border systolic murmur was present. The second sound was loud though not audibly split, and a diastolic rumble was heard at the apex. In April 1959, her findings were similar. X-rays now showed biventricular enlargement and a prominent left atrium. There was increased pulmonary vascularity suggestive of a persisting left-to-right shunt. Surgery was again performed in August 1959 and the reopened defect was identified. Closure was effected by means of interrupted mattress sutures reinforced by compressed Ivalon. In the immediate postoperative period, a third sound was heard at the apex and the liver was enlarged. The patient was digitalized and placed on a salt-free diet. The liver regressed in size, and the patient improved rapidly. At the time of discharge the precordium was quiet, and a residual grade II systolic murmur was heard at the left sternal border.

#### Discussion

##### Description of Patient and Preoperative Status

The case reports include seven patients ranging in age from 8½ to 35 years. They all had ventricular septal defects. Six had complicating infundibular stenosis or a greatly hypertrophied crista supraventricularis. One patient was cyanotic. Two of the patients with pulmonary stenosis were asymptomatic. The remainder of the group had degrees of disability ranging from mild exertional dyspnea to easily induced fatigue, breathlessness, or cyanosis. Three patients had experienced sub-

normal growth and development. None had experienced congestive heart failure preoperatively although one child had been on salt restriction and digitalis because of "pulmonary congestion."

#### Description of Defects and Technic of Closure

All the defects were located in the region of the membranous septum, but were not necessarily delimited by the position of this structure. They were most commonly bordered by the insertion of the tricuspid valve below and by the aortic valve in front. Although one of the defects was described as a 3 to 4 mm. slit in systole, the remainder varied from 1.5 to 2.5 cm. in diameter. Characteristically, the defects lacked a firm fibrous rim, the edge of the defect consisting instead of cardiac muscle.

The defects were repaired by conventional methods, and an Ivalon prosthesis was usually employed to close the defect or reinforce the closure. The infundibular stenosis was relieved by resection of muscle in the outflow tract and crista supraventricularis.

#### Time of Detection of Persisting Shunt

This could not be ascertained precisely in each instance. In three cases findings suggestive of a persisting shunt were present on the first to third postoperative day. In the other cases, the significant auscultatory observations were not made until the fifth to eighth day. Significantly, in no case did earlier auscultatory findings, indicating the closure of a shunt, precede notes reporting the reappearance of a shunt. It is probable that in all cases the defect was either never completely closed or that the reopening occurred within the first few postoperative days. This has also been the experience elsewhere.<sup>10, 11</sup>

#### The Syndrome of Persisting Shunt

It will be noted above that, preoperatively, the symptomatology of these patients was quite variable, ranging from no symptoms to easily induced dyspnea. In none, however, was dyspnea present at rest, and none had orthopnea or objective evidence of heart failure. This relatively benign situation was profoundly altered by the persistence postopera-

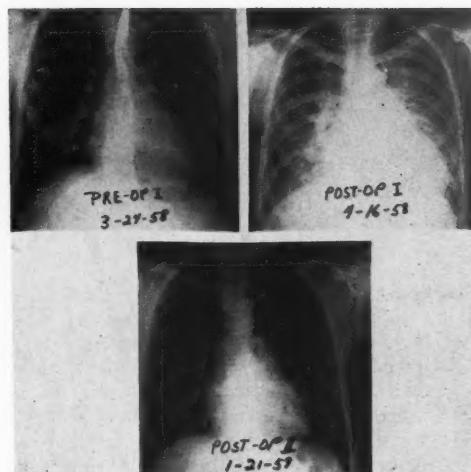


Figure 1

*J.L., an 11-year-old boy with interventricular septal defect and infundibular stenosis. Upper left. The heart is moderately enlarged preoperatively, and other projections confirm that the enlargement involves the right ventricle mainly. The pulmonary vascular markings are moderately accentuated suggesting a modest left-to-right shunt. Upper right. Postoperatively the defect is still open but the infundibular stenosis has been removed. There is an increase in heart size due to biventricular enlargement. The pulmonary arterial branches are full, suggesting a large left-to-right shunt, and the central vascular engorgement indicates edema of the lung. Lower. After successful closure of the defect, the cardiac silhouette has become small and the lungs show no evidence of increased flow or congestion.*

tively of a left-to-right shunt. Sinus tachycardia and tachypnea were the rule, and these became increasingly significant after the first 72 hours, when fluid balance had been stabilized and when postoperative pain or fever had been excluded. The patient was usually dyspneic and orthopneic. The pulses were small. The precordium was hyperactive. There was a right ventricular heave; occasionally, a left ventricular heave. At this time a thrill and a full systolic murmur were present at the lower sternal border. If right bundle-branch block had occurred, the second sound was broadly split. A third sound or diastolic flow murmur, not heard preoperatively, could be detected in three instances. All these find-

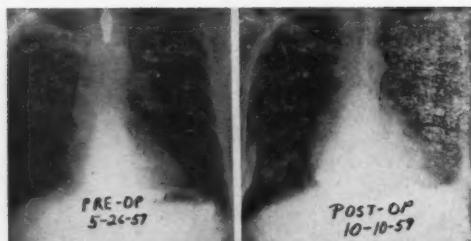


Figure 2

*Ro.Ja.*, a 26-year-old man with interventricular septal defect and infundibular stenosis. Left. The heart is moderately enlarged and the lung fields suggest a small increase in pulmonary blood flow. Right. Postoperatively there is distinct broadening of the cardiac silhouette and the pulmonary vasculature is more prominent, suggesting an increase in left-to-right flow after failure to close the defect at a time when the infundibular stenosis had been removed. The right atrium and superior vena cava appear dilated in this patient, who had signs of tricuspid insufficiency.

ings might be present in the early postoperative period. Later pulmonary rales and edema, distended neck veins, hepatic enlargement, and dependent edema were likely to appear.

The most striking changes appeared in the five patients who had had significant infundibular gradients as well as ventricular septal defects preoperatively. The pulmonary stenosis had been adequately relieved in these instances and, in some, the left-to-right shunt now seemed to be greater than before surgery. This was suggested by the strikingly dynamic precordial movements, by the appearance for the first time of an apical third sound or flow murmur, or by the murmur of pulmonary insufficiency. Notable too was the appearance of tricuspid insufficiency in at least three of this group. This was signified by systolic jugular pulsations followed by rapid diastolic collapse, and by the pronounced evidence of right-sided failure, which included high venous pressure, hepatomegaly, edema, and ascites. Because of the loud murmur of the ventricular septal defect, the presence of a discrete murmur of tricuspid insufficiency was difficult to establish but was identified in at least one instance where it was characteristically louder on inspiration. In this individ-

ual the liver was pulsatile. Pulmonary congestion also occurred, and one patient had frank pulmonary edema indicating that left ventricular failure is a distinct part of this syndrome. Five of the patients had to be treated intensively with digitalis, salt restriction, diuretics, and, occasionally, oxygen. One patient had atrial fibrillation which was later converted to sinus rhythm.

#### Effects of the Persisting Shunt on the X-ray and Electrocardiogram

The cardiac silhouette regularly increased in size in the presence of a persisting left-to-right shunt. This was quite striking since, by comparison, the preoperative increases in the cardiac silhouette were usually modest. Figures 1 and 2 illustrate this point. There were also notable changes in the lung fields. For example, in both figures the lungs are more vascular postoperatively, indicating greater pulmonary blood flow. There is also hilar engorgement and diffuse haziness in figure 1, which was taken after the patient had experienced pulmonary edema. In figure 2, there is dilatation of the right atrium and superior vena cava, and this film was taken at a time when clinical and hemodynamic evidence of tricuspid insufficiency was present. In oblique views some enlargement of the left atrium is also apparent.

Electrocardiographic alterations are more difficult to assess, since the electrocardiogram is often altered by surgery alone. Electrocardiograms before and after operation were available in six cases. In one instance the preoperative tracing remained that of complete right bundle-branch block postoperatively. Complete right bundle-branch block appeared in three cases where patterns of right ventricular hypertrophy were originally present and in two cases where, preoperatively, the QRS duration was less than 0.12 second and an *rsr'* or *rsR'* complex was present in *V<sub>1</sub>*.

#### Cardiac Catheterization Data

Preoperatively left-to-right shunts in the patients with interventricular septal defects complicated by infundibular stenosis were

not large, and in only one instance did the pulmonary to systemic flow ratio exceed 2.3/1. One patient was cyanotic and had no net left-to-right shunt preoperatively. Systolic pressure in the right ventricle ranged between 79 and 117 mm. Hg, and the peak systolic gradient between the main right ventricular chamber and the pulmonary artery ranged between 65 and 87 mm. Hg, indicating moderate infundibular stenosis. With one exception the right ventricular diastolic and mean right atrial pressures were normal.

Postoperative cardiac catheterization invariably disclosed the presence of a left-to-right shunt, whereas the infundibular gradient had been eliminated or reduced. Of the six patients with preoperative gradients, postoperative catheter data showed no gradient in two, and residual systolic gradients of 49 and 15 mm. Hg, respectively, in two others. In the two remaining cases, observations at second operation suggested that the obstruction had been successfully eliminated.

The pulmonary to systemic flow ratios were either essentially unchanged or greater postoperatively. At times the increase in pulmonary blood flow was striking. For example, in table 1 are the data on an 11-year-old boy with so-called acyanotic tetralogy. He had a modest left-to-right shunt preoperatively, the pulmonary to systemic flow ratio being just less than 2/1, and there was a peak systolic gradient of 87 mm. Hg between the right ventricle and pulmonary artery. Postoperatively this gradient was eliminated. At the same time the pulmonary blood flow has become very large, but it can only be approximated under these circumstances because of the difficulty in sampling true mixed venous blood. If a pulmonary arteriovenous difference of 1.0 ml. per 100 ml. is assumed, the estimated pulmonary blood flow is 29 liters per minute. The mild arterial desaturation is probably due to hypoventilation induced by presedation with secobarbital and meperidine. The pulmonary wedge pressure was 24 mm. Hg, and although a flow gradient across the mitral valve cannot be excluded, it is probable that there was also an elevation

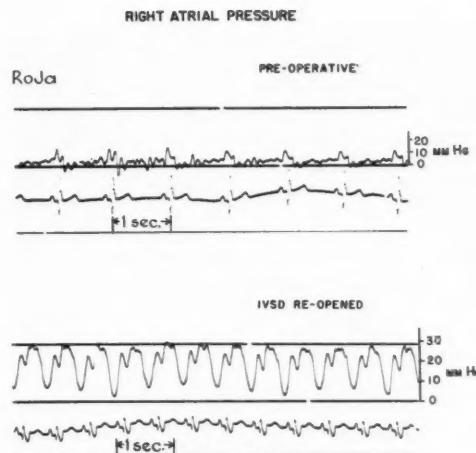


Figure 3

Same patient as in figure 2. The right atrial tracing was not remarkable preoperatively. With the interventricular septal defect still open postoperatively, the right atrial pressure is typical of tricuspid insufficiency. Note especially the merged systolic c-v peaks and the rapid y descent.

of left ventricular diastolic pressure. Right ventricular diastolic pressure was clearly elevated. Right atrial pressure curves of course reflected these ventricular events, but at times they also offered graphic evidence of tricuspid insufficiency. This is illustrated in figures 3 and 4.

#### Reoperation to Close the Persisting Shunt

In all instances a second attempt was made to close the defect. The second operations were performed at a minimum of 4½ weeks after the first and in all but one instance within 6 months. In one case a third closure was attempted 5 months after the second operation. There were two postoperative deaths. One was due to intractable intrathoracic hemorrhage, oliguria, and hypotension in a patient who had had severe tricuspid insufficiency and heart failure after the second operation. The second death occurred in a patient who had staphylococcal endocarditis at the site of his repair, osteomyelitis, and heart failure. This patient survived a second operation but died in heart failure after the third operation.

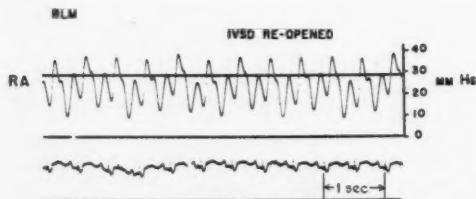


Figure 4

B.L.M., a 17-year-old boy with tetralogy of Fallot, The postoperative pressure curve from the right atrium is indicative of tricuspid insufficiency. The curve is "ventricularized" during systole and there is a rapid y descent.

The most constant finding at reoperation was the detachment of the Ivalon and Teflon prostheses. There was an apparent failure of tissue to invade the material and create a firm union when it was used as a supporting plug. In addition, where the material had been retained by sutures and became firm, it tended to fracture at the suture line, suggesting brittleness. There was also a single Teflon cloth failure apparently due to inadequate invasion of the material from the margin of the repair.

#### Improvements in Surgical Technique

During the past 2 years, we have repaired the ventricular defect with interrupted sutures of number 3-0 silk. The repair is then covered with a crimped Dacron patch held in place with interrupted sutures. Great care is taken with the lower margin, where recurrences are prone to occur. Here some of the redundant tricuspid valve is often drawn in with the first suture. Since using this method, recurrences have virtually disappeared.

Potassium arrest was abandoned approximately 2 years ago in favor of general body hypothermia in conjunction with extracorporeal circulation. For tetralogy of Fallot or complicated interventricular defects, temperatures of 20 to 24 C. are reached through the use of a heat-exchange unit incorporated in the lung. At approximately 26 C., the heart fibrillates, and at lower temperatures, it often will cease beating. When necessary, the aorta is cross-clamped for short periods of time. Left atrial decompression is routinely em-

ployed with a catheter placed in the right side of the left atrium.

General body hypothermia has been combined with extracorporeal circulation in over 240 cardiac procedures of which 110 were interventricular defects of tetralogy of Fallot.<sup>16</sup> Permanent heart block has virtually disappeared since employing the above technique. In only one operation for tetralogy of Fallot was death probably caused by this complication, and there is only one living patient with permanent block.

#### Conclusions

Disruption of a ventricular septal defect repair is particularly, though not exclusively, a complication in the open cardiotomy treatment of patients with coexisting infundibular stenosis. The disruption occurs within the first few days and may usher in profound circulatory disturbances. As a consequence patients who were relatively asymptomatic exhibit symptoms and signs of biventricular failure and tricuspid insufficiency. An anatomic basis for this is provided by the successful elimination of, or significant reduction of, the infundibular obstruction. The example cited above and in table 1 indicates how, under these circumstances, a patient who had had a relatively small left-to-right shunt may develop an extremely large pulmonary blood flow approximating 30 liters per minute. Such a flow is clearly larger than would be customary for a ventricular septal defect in adults. The estimated pulmonary vascular resistance of 0.5 units is low; this suggests the failure of the pulmonary vasculature to regulate flow through vasoconstriction.

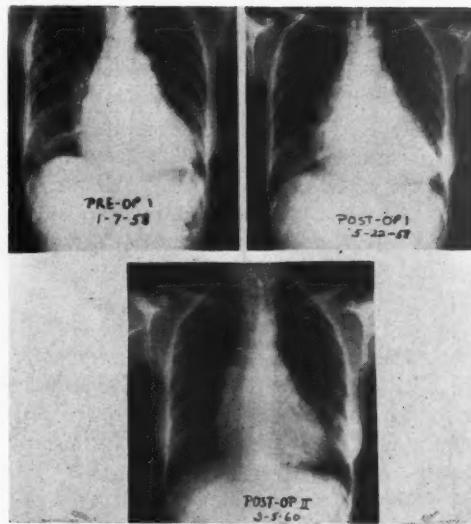
However, tremendous increases in pulmonary blood flow were not always present and cannot be solely responsible for the production of this syndrome. In the case represented in table 3, for example, the pulmonary blood flow has increased by a third only, probably because residual infundibular obstruction was present, and because the pulmonary vascular resistance increased. The patient whose data are shown in table 2 exhibited the features of this syndrome even though her repair was only partially disrupted so that she had the

combined benefits of an actual reduction in pulmonary flow and of the complete elimination of infundibular stenosis. In such individuals it would appear that something had happened relatively acutely so that the heart was now unable to handle a work load that for many years it had supported with few ill effects.

In this regard some evidence has accumulated to suggest that the methods employed for cardiac arrest at surgery and ventriculotomy itself may have an adverse effect on the myocardium. In most of these repairs potassium arrest was used. It has recently been shown in acute experiments that potassium arrest depresses left ventricular function considerably.<sup>17</sup> Furthermore, the effects of right ventriculotomy done in the conventional way from base to apex along the long axis of the ventricle are not entirely benign. Ventricular function curves<sup>18</sup> and high-speed cinemicrofilms<sup>19</sup> before and after right ventriculotomy in experimental animals have shown appreciable depression of function and alterations in the sequential contraction pattern of the right ventricle. These alterations in function can persist after a number of months.<sup>19</sup> Such a ventricle may perform adequately when not stressed but could fail when presented with a persisting left-to-right shunt postoperatively.

The tendency for disruption to occur in patients with infundibular stenosis is understandable. The defect is in close relation to the area of stenosis and to the thickened, overgrown crista supraventricularis, making exposure suboptimal. In spite of resection of the obstructing muscle elements, the edges of the defect may be difficult to bring together easily without tension. Moreover, the tough fibrous edge, which is the rule in uncomplicated ventricular septal defects, is not present, and the margin of the defect consists of soft cardiac muscle, which is not so suitable for holding sutures.

A major disappointment has been the failure of Ivalon and Teflon prostheses in our hands. We have observed disruptions of the suture line and failure of the material to be



**Figure 5**  
*F.H., a 37-year-old woman with interventricular septal defect and infundibular stenosis. The first postoperative film, upper right, shows continuing cardiac enlargement and persistence of full lung fields. The second operation successfully closed the defect. The lower film taken 6 months later shows very considerable reduction in cardiac size and the pulmonary vasculature is normal.*

overgrown by tissues, the requisite of a firm repair. There has also been some evidence of brittleness of the material. These experiences have led to the adoption of the present method, which consists in direct suture of the defect with interrupted silk, followed by the application of a crimped Dacron patch over the repair with interrupted sutures. Potassium arrest has been discontinued, and cardiac arrest or ventricular fibrillation is now induced by total body hypothermia routinely in the treatment of tetralogies and large ventricular septal defects. The present approach seems promising, and there have been no recurrences or deaths in the last 25 cases of tetralogy of Fallot.

#### Summary

The persistence of a left-to-right shunt due to the unsuccessful repair of a ventricular septal defect associated with infundibular stenosis has been described.

A characteristic clinical picture has been reported. The main features include congestive heart failure, tricuspid insufficiency, and increased pulmonary blood flow.

The severity of the symptoms appears to be due to the persistence of a left-to-right shunt at a time when the myocardium has been affected adversely by such operative insults as potassium arrest and ventriculotomy.

The particular difficulties attending the repair of a ventricular septal defect with infundibular stenosis include the proximity of the defect to the hypertrophied muscle mass, the soft muscular margin of such a defect, and the tension on the suture line. Ivalon and Teflon prostheses have been disappointing in their failure to maintain the integrity of the repair.

All of the patients were reoperated upon. Five survived and their defects are now closed. The second repairs were abetted by the development of a fibrous scar around the margin of the defect. The five survivors are in good health and there is no evidence of a remaining shunt.

As a consequence of these experiences, surgical techniques were modified. The defects are now closed by direct suture with interrupted silk, followed by the application of a crimped Dacron patch over the repair. The results have been encouraging and since the new technic has been adopted, there have been virtually no recurrences.

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## Systemic and Pulmonary Emboli before and after Mitral Commissurotomy

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**S**YSTEMIC AND PULMONARY emboli are frequent and serious complications<sup>1-3</sup> in mitral stenosis. It has been stated<sup>4, 5</sup> that systemic embolism occurring in patients with mitral stenosis is an indication for mitral commissurotomy, and surgery has been recommended<sup>6, 7</sup> as an urgent or emergency procedure in patients with recent systemic embolism to prevent more episodes.

In one series<sup>8</sup> of 50 patients followed for 4½ to 7 years after commissurotomy no post-operative emboli were encountered. Others have noted systemic emboli during the first 2 years after commissurotomy<sup>1, 6, 9, 10</sup> but some<sup>8, 10</sup> have thought that the incidence was less than the preoperative occurrence.

In contrast to these reports, we have been impressed with the frequency of embolic phenomena following mitral commissurotomy; so we were prompted to investigate the relation of mitral commissurotomy to postoperative emboli.

### Material and Methods

This study includes 149 patients who had mitral commissurotomy from 1950 to 1959 at Los Angeles County Harbor General Hospital, Torrance, and Long Beach Veterans Administration Hospital. It includes all the 75 patients operated upon at the Harbor General Hospital and 74 of 80 patients at the Veterans Hospital.\* There were 91 male and 58 female patients. Four were in the second decade, 18 in the third, 53 in the fourth, 53 in the fifth, and 21 in the

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\*The records of six patients were unavailable.

sixth and seventh decades. According to the functional classification of the American Heart Association, six were in class I, 50 in class II, 82 in class III, nine in class IV, and two were unclassified.

Mitral stenosis was considered marked if the estimated valvular area at the time of surgery was less than 1 cm.<sup>2</sup>, moderate if the estimated area was from 1 to 1.5 cm.<sup>2</sup> and slight if the estimated area was from 1.5 to 2 cm.<sup>2</sup> One hundred ten patients had marked, 19 had moderate, and five had slight mitral stenosis. In 12 others the degree of stenosis was not mentioned. Three were found to have pure regurgitation. Mitral commissurotomy was considered adequate if the estimated opening of the mitral valve was larger than 2.5 cm.<sup>2</sup>

Mitral regurgitation was considered marked if the regurgitant jet was estimated as greater than 10 ml.; moderate if 5 to 10 ml.; and mild if less than 5 ml. at surgery. Ten patients had marked regurgitation, 21 had moderate degree, 30 had slight insufficiency, but 75 had no regurgitation. Information was not available on 13 patients. After mitral surgery, mitral regurgitation was marked in 17, moderate in 32, slight in 26, and absent in 56. Information was not available on 18 patients. In no instance was the degree of regurgitation decreased by surgery.

Prior to surgery, 76 of the 159 patients had sinus rhythm, and 73 had atrial fibrillation. Of the 136 patients surviving surgery, 45 maintained sinus rhythm and 89 had atrial fibrillation.\*

Commissurotomy was considered adequate in 119 patients and inadequate in 15 patients. Information was not available in 15 patients.

The diagnosis of systemic emboli was based on the clinical symptoms and signs of cerebral, peripheral arterial, or visceral emboli (table 1). Cerebral emboli were manifested by unilateral motor paralysis except in two instances in whom right homonymous hemianopsia developed. Peripheral arterial emboli were characterized by sudden onset of pain, with absent pulse in the affected artery, and was verified in the majority by either arterial embolectomy or

\*Information unavailable in 2 patients.

Table 1  
Systemic Emboli

	Cerebral	Peripheral or abdominal arteries	Visceral	Total
Preoperative	38	15	9	62
Operative	8	1	0	9
Postoperative	16*	3	2	21

\*One patient also had renal infarction found at autopsy.

amputation. Visceral emboli to the kidney or spleen were indicated by severe pain in the regions of affected organs with transient hematuria in the case of kidney emboli. Postmortem examination verified splenic emboli in three patients and renal emboli in two.

Pulmonary emboli were manifested by the symptoms and signs of pulmonary infarction.

#### Results

There were 13 surgical deaths in the series of 149 patients with a surgical mortality rate of 8.7 per cent. Nine patients, four of whom had had preoperative systemic emboli, had systemic embolization associated with surgery, an operative embolic incidence of 6 per cent (table 2). Four with systemic emboli died and five survived.

The survivors were followed for an average of 3.2 years after surgery with a maximum of 9 years. In 23 patients the follow-up was less than 1 year. There were 11 (8.1 per cent) late deaths in the 136 surviving patients, with four of these dying of cerebral embolism and four dying of pulmonary infarction and congestive heart failure.

Prior to surgery (table 2), 42 of the 149 patients, or 28 per cent, had a total of 62 systemic emboli. Twenty-eight patients had a single episode, 11 patients had two, and the other three patients had three, four, and five episodes respectively. Sixteen (28 per cent) of the 56 patients in functional classification I and II, 24 (25 per cent) of the 91 patients in classes III and IV, and two unclassified patients had systemic emboli prior to surgery. Thirteen (table 3), or 17 per cent, of the 76 patients with sinus rhythm and 29, or 40 per cent, of the 73 patients with atrial fibrillation had preoperative systemic emboli.

Postoperatively, 17 (12.5 per cent) of the 136 surviving patients had 21 late systemic emboli. Thus the incidence of late systemic emboli was 4.8 per cent per patient-year during the follow-up period. Twelve of these 17 patients had systemic emboli after surgery for the first time. Inclusion of the operative emboli made a total of 30 episodes of systemic emboli in 25 patients, or an incidence of 18.5 per cent.

Of the surviving patients, 11 were in functional class I, 80 in class II, 37 in class III, seven in class IV; and one was unclassified. Nine (10 per cent) of the 91 in classes I and II and eight (17 per cent) of the 44 in classes III and IV had postoperative systemic emboli. Five patients (table 3) or 11 per cent of the 45 with sinus rhythm and 12 patients or 13.5 per cent of the 89 with atrial fibrillation had subsequent embolic episodes. Of the 17 patients with postoperative systemic emboli, 13 (85 per cent) had an adequate mitral commissurotomy, and three had inadequate surgery. Information was not available on one patient.

The 12 patients who had systemic emboli for the first time following commissurotomy ranged in age from 19 to 59. Ten were in functional class II, one in III, and one in IV; eight had predominant mitral stenosis and four had significant insufficiency; eight were noted to have calcification of the valve. Eleven were considered to have an adequate commissurotomy and five of the 12 were clinically improved one functional class following surgery. Three had atrial fibrillation prior to surgery; six had this arrhythmia following surgery, and three continued to have sinus rhythm following surgery.

Thirty-two (table 4) (21 per cent) of the 149 patients had atrial thrombi at surgery. Nine (28 per cent) of these 32 patients had preoperative systemic embolic episodes; two had operative, and two had postoperative systemic emboli.

Eighty-one (table 4) (54 per cent) of the 149 patients had a calcified mitral valve. Twenty-three (28 per cent) of these 81 pa-

Table 2  
*Systemic Emboli before, during, and after Surgery*

		Number of patients	Number of emboli					Total
			1	2	3	4	5	
Preoperative emboli (149 Patients)	Number of patients	28	11	1	1	1	42	
	Total number of emboli	28	22	3	4	5	62	
Operative emboli (149 Patients)	Number of emboli or patients		9				9	
Postoperative emboli (136 Patients)	Number of patients	13	4	—	—	—	17	
	Total number of emboli	13	8	—	—	—	21	

Table 3  
*Relation of Systemic Embolization to Cardiac Rhythm*

	Rhythm	Number of patients	Number of systemic emboli	Per cent of patients
Preoperative, 149 patients	Sinus atrial fibrillation	76 73	13 29	17 40
Postoperative, 134 surviving patients*	Sinus atrial fibrillation	45 89	5 12	11 13.5

\*Information on rhythm postoperatively unavailable in two patients.

tients had preoperative systemic emboli; three had operative, and 10 had postoperative systemic emboli.

Ten patients (6 per cent) had pulmonary infarction prior to surgery and 10 (7.3 per cent) had pulmonary infarction after surgery. Four of the latter died of pulmonary infarction and congestive heart failure in the follow-up period.

#### Discussion

The incidence of systemic emboli prior to surgery of 28 per cent is higher than that of many series but is comparable with the observations of Olesen<sup>2</sup> on the natural history of mitral stenosis.

The incidence of systemic emboli complicating surgery of 6 per cent is significant and is comparable to the rates of 2 to 8.4 per cent reported by others.<sup>4, 10, 11</sup> Anticoagulant therapy begun prior to surgery and continued through the immediate postoperative period has been shown<sup>3, 12</sup> to decrease this surgical complication.

Following surgery the incidence of sys-

temic embolization is less than the preoperative incidence but represents 4.8 per cent per patient-year, which is eight times the incidence reported by Ellis.<sup>6</sup> It is difficult to determine the preoperative incidence of emboli per patient-year but if one arbitrarily assumed 10 years as a preoperative period of vulnerability, our preoperative incidence would have been 3 per cent per patient-year, which is comparable to the incidence in Olesen's series<sup>2</sup> of 4 per cent per patient-year. (Several of our patients had emboli 6 years prior to surgery and one had an embolus 9 years before.)

It is also noteworthy that 12 patients had their initial systemic emboli following surgery. Thus our findings would suggest that mitral commissurotomy neither prevents nor decreases the incidence of systemic embolization.<sup>13</sup> It has been reasonably hoped that the improved circulatory hemodynamics following adequate commissurotomy would decrease the incidence of systemic emboli. Perhaps the endothelial defect is more important in the production of mural thrombi than a reduced

**Table 4**  
*Incidence of Systemic Embolization in Patients with Atrial Thrombosis or Calcified Valve*

	With systemic emboli			Without systemic emboli	Total
	Preoperative	Operative	Post-operative		
Left atrial thrombosis	9 (28%)	2	2	19	32
Calcified mitral valve	23 (28%)	3	10*	51	81

\*Five of the 10 patients also had preoperative systemic emboli.

flow across the mitral valve. Unless there are other indications for mitral commissurotomy, systemic embolization may best be prevented with prolonged anticoagulant therapy.<sup>14</sup>

Atrial fibrillation appears to predispose to systemic emboli before commissurotomy.<sup>15</sup> Following surgery the incidence of emboli was less in functional classes I and II than in III and IV, but prior to surgery the incidence was essentially the same in the two groups.

Contrary to a published report,<sup>16</sup> the presence of atrial thrombosis or calcified mitral valve was not accompanied by an increased incidence of preoperative systemic emboli.

The observations concerning pulmonary infarction suggest that mitral commissurotomy has little effect on the incidence of pulmonary emboli, which probably occur as late manifestations in the course of rheumatic heart disease.

#### Conclusion

In a series of 149 patients subjected to mitral commissurotomy, 28 per cent had preoperative systemic embolization. Nine patients, or 6 per cent, had systemic emboli associated with surgery. Of the 136 patients surviving surgery, 12.5 per cent had subsequent systemic emboli with an incidence of 4.8 per cent per patient-year, comparable to the reported natural incidence of systemic emboli of 4.0 per cent per patient-year.

This study suggests that mitral commissurotomy does not prevent systemic emboli and does not decrease the natural incidence of such emboli.

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# Left Atrial and Left Ventricular Pressures in Subjects without Cardiovascular Disease

## Observations in Eighteen Patients Studied by Transseptal Left Heart Catheterization

By EUGENE BRAUNWALD, M.D., EDWIN C. BROCKENBROUGH, M.D.,  
CHARLES J. FRAHM, M.D., AND JOHN ROSS, JR., M.D.

THE MEASUREMENT of pressures in the left side of the heart now constitutes one of the basic technics in the clinical study of the circulation. Left heart catheterization is of importance not only in cardiovascular diagnosis but also as a tool in the physiologic investigation of the central circulation in normal and abnormal states. In spite of the widespread applications of left heart catheterization, the level of pressures in the left side of the heart in subjects without cardiovascular disease in a basal physiologic state has not been known. The lack of this information is understandable when the risk, patient discomfort, and technical complexity associated with the older methods of left heart catheterization are considered.<sup>1</sup> These procedures were in general reserved for those patients in whom the establishment of a specific diagnosis or of a therapeutic plan required specific knowledge of the pressures in the left side of the heart.

With the development of left heart catheterization by the transseptal route<sup>2-5</sup> it has become possible to study the dynamics of the left side of the heart with relative safety and little discomfort to the patient. We have had the opportunity to measure left atrial and left ventricular pressures in 18 subjects without any apparent abnormalities of the cardiovascular system; the data obtained in these patients form the basis of this report.

### Methods

The subjects studied ranged in age from 5 to 49 years, with an average age of 21 years; 11 of

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them were male and 7 were female. All were studied because of the presence of heart murmurs. On clinical examination these murmurs were considered to be functional in origin by several examining physicians, and the chest roentgenograms and electrocardiograms showed no abnormalities. Right heart catheterization was carried out through the right saphenous vein, and in each instance the pressures in the pulmonary artery, right ventricle, and right atrium were found to be within normal limits.<sup>6</sup> There was no evidence of a circulatory shunt by application of indicator-dilution curves<sup>7</sup> and of foreign gas techniques.<sup>8</sup> Following right heart catheterization transseptal left catheterization was carried out in a manner detailed previously.<sup>4, 5</sup>

Left atrial pressures were measured through a no. 17-gage thin-walled needle in 16 patients and through a no. 19-gage needle in the other patients. Left ventricular pressures were measured through a polyethylene catheter (PE no. 50), 100 cm. in length in 16 patients, and through a radiopaque polyethylene catheter, 70 cm. in length with an internal diameter of 1.15 mm. in the other two patients. Pressures were measured with P23D Statham pressure transducers and were recorded on a multi-channel photographic recorder. The baseline for all pressure measurements was 5 cm. below the sternal angle.

All patients were studied in the basal, postabsorptive state. Thirteen of them were given 100 mg. of pentobarbital orally while the five children, aged 5 to 14 years, received a mixture of meperidine, phenergan, and promazine intramuscularly prior to study.

### Results

The results are presented in detail in figure 1. The mean left atrial pressures ranged between 2 and 12 mm. Hg, and the average value was 7.9 mm. Hg. The mean left atrial pressure exceeded the mean right atrial pressure in every subject; the difference between these mean pressures ranged from 1 to 7 mm. Hg, and the average difference was 3.9 mm.

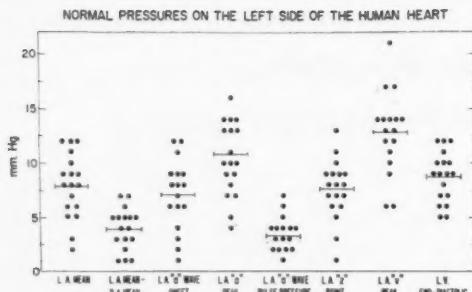


Figure 1

*Summary of observations of pressures on the left side of the heart of subjects without cardiovascular disease. Each point represents an observation on one patient. The horizontal bar represents the mean value for each series of measurements. L.A., left atrium; R.A., right atrium; L.V., left ventricle.*

Hg. The left atrial pressure at the onset of the atrial contraction (a) wave was, in general, almost identical to the mean left atrial pressure, ranging from 1 to 12 mm. Hg, with an average value of 7.1 mm. Hg. The left atrial a wave peak ranged from 4 to 16 mm. Hg and averaged 10.4 mm. Hg. Thus, the left atrial a wave pulse pressure, i.e., the difference between the pressure at the onset and at the peak of the a wave ranged from 1 to 7 mm. Hg and averaged 3.4 mm. Hg. The left atrial z point pressure,<sup>9</sup> i.e., the atrial pressure at the onset of left ventricular contraction, ranged from 1 to 13 mm. Hg and averaged 7.6 mm. Hg. The tallest wave in the left atrial pressure pulse was generally the v peak, i.e., the pressure at the time of the opening of the mitral valve. This ranged from 6 to 21 mm. Hg and averaged 12.8 mm. Hg. The left ventricular end-diastolic pressure differed little from the mean left atrial and the left atrial z point pressures; it ranged from 5 to 12 mm. Hg, with an average value of 8.7 mm. Hg.

A typical left atrial pressure pulse in one of the subjects is reproduced in figure 2.

#### Discussion

It is well established, on the basis of experimental observations in the dog, that the mean pressure in the left atrium normally exceeds that in the right atrium.<sup>10</sup> Although pressures from the left atrium have been re-

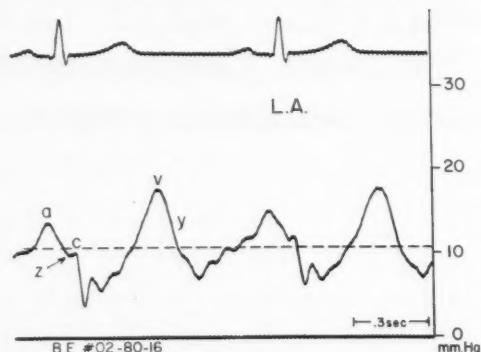


Figure 2

*A representative left atrial (L.A.) pressure tracing.*

corded in patients with atrial septal defects at the time of cardiac catheterization for many years,<sup>11, 12</sup> the presence of the interatrial communication in such patients lowers the left atrial pressure and reduces the normal interatrial pressure gradient. Thus, the left atrial pressure in these patients cannot be considered to be representative of that existing in normal subjects. Left heart pressure measurements in patients without cardiovascular disease have, up to now, been limited to observations carried out at the time of thoracotomy.<sup>13-17</sup> In patients with an open chest the mean left atrial pressure averaged 7.5 mm. Hg in one series<sup>15</sup> and 9.0 mm. Hg in another;<sup>16</sup> the left ventricular end-diastolic pressures ranged from 5 to 14 mm. Hg in one group<sup>15</sup> and 5 to 17 mm. Hg (mean 9 mm. Hg) in the others.<sup>16</sup> The mean left atrial pressure exceeded the mean right atrial pressure by an average of 2 mm. Hg, whereas the left ventricular end-diastolic pressure exceeded the right ventricular end-diastolic pressure by an average of 3 mm. Hg.<sup>16</sup> The close correspondence between these values, obtained at the time of operation, and the pressure values obtained at catheterization and reported herein is of interest.

#### Summary

Transseptal left heart catheterizations were carried out in 18 patients without apparent evidence of organic cardiovascular disease.

These studies have permitted delineation of the pressures that exist in the left side of the heart in normal subjects studied in a basal physiologic state.

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There is no disease more conducive to clinical humility than aneurysm of the aorta.—  
SIR WILLIAM OSLER. *Aphorisms From His Bedside Teachings and Writings*. Edited by William Bennett Bean, M.D. New York, Henry Schuman, Inc., 1950, p. 134.

## Aneurysm of the Distal Popliteal Artery and Its Relationship to the Arcuate Popliteal Ligament

By STAFFORD W. GEDGE, M.D., JOHN A. SPITTEL, JR., M.D.,  
AND JOHN C. IVINS, M.D.

IN 1953 Gifford, Hines, and Janes<sup>1</sup> clearly demonstrated the high incidence of serious complications in patients with untreated aneurysms of the popliteal artery. They pointed out that arteriosclerosis was present in almost all the 100 popliteal aneurysms that they studied and that the aneurysms were not infrequently bilateral or multiple.

Popliteal aneurysms are seen predominantly in men more than 50 years of age and are usually asymptomatic until complications occur. These may be local swelling and pain, a prominent venous pattern or edema below the knee, or ischemia with intermittent claudication, ischemic neuritis, ulceration, and gangrene. The complications are results of local pressure from or rupture of the aneurysm, thrombosis within the aneurysm, or peripheral embolization from the aneurysm.

In 1916 Halsted<sup>2</sup> and Reid<sup>3</sup> experimentally produced circumcribed dilatation of an artery immediately distal to a partially occluding band and related this phenomenon to the dilatation of the subclavian artery observed in certain cases of cervical rib. Similar post-stenotic dilatation has often been observed in other arteries. In the aorta it appears commonly distal to stenosed aortic and pulmonary valves and distal to coarctate segments. The physical phenomena that occur distal to a stenotic segment of artery were described by Holman<sup>4</sup> in 1954 as follows:

A mass of fluid ejected through a narrow and limited constriction under high velocity strikes against a more slowly moving mass of fluid distal to the stenosis, resulting, first, in the conversion of high kinetic energy into high potential energy or lateral pressure and, second, in the lateral deflection of the rapid stream and even in a complete reversal in the direction of flow, thus producing

eddies of alternating high and low pressure whose repeated impacts over prolonged periods against an elastic wall are capable of inducing structural fatigue and distention of that wall, resulting eventually and inevitably in the phenomenon of poststenotic dilatation.

To relate the phenomenon of post-stenotic dilatation to the pathogenesis of popliteal aneurysms, a review of the anatomy of the popliteal space is necessary (fig. 1). About two thirds of the way down the thigh, the femoral artery passes posteriorly and inferiorly through the tendinous hiatus of the adductor magnus and enters the popliteal fossa as the popliteal artery. Within the popliteal space the popliteal artery lies in loose fatty tissue and is freely mobile. According to Boyd and co-workers,<sup>5</sup> the popliteal artery then enters a fibrous tunnel derived from the fascia on the deep surface of the gastrocnemius just above the level of the knee joint. The fascial covering narrows to form a definite fibrous band,  $\frac{1}{4}$  to  $\frac{1}{2}$  inch broad, attached to the capsule of the knee joint at the level of the joint.

In addition to the fibrous band described by Boyd, which is posterior to the popliteal artery, there is another ligamentous structure, the arcuate popliteal ligament, which is anterior to the popliteal artery. The arcuate popliteal ligament arches upward on the lateral side of the popliteus muscle from the head of the fibula, crossing the popliteus muscle and blending into the ligaments of the posterior knee joint medially. This ligament is particularly sharp and prominent when the lower leg is fully extended.

The popliteal artery crosses the arcuate popliteal ligament posteriorly at the level of the knee joint or just inferiorly. It is at this point that arteriograms have shown thrombosis of the popliteal artery most often.<sup>5</sup> As

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a corollary, it is distal to this point that popliteal aneurysms may occur. It is conceivable then that popliteal aneurysms may result not only from post-stenotic dilatation distal to the adductor magnus hiatus but also from post-stenotic dilatation distal to the arcuate popliteal ligament.

It should also be recognized that the popliteal fossa is a rather confined space covered with the strong sural fascia. Pressure applied posteriorly to the popliteal artery would compress the posterior tibial nerve and popliteal vein before the artery itself. Therefore it is hypothesized that in distal popliteal aneurysm the pressure resulting in post-stenotic dilatation of the artery arises anteriorly from the arcuate popliteal ligament, rather than posteriorly from a fibrous band as described by Boyd. Distal to the arcuate popliteal ligament the popliteal artery is cushioned by the popliteus muscle, and the artery terminates close to the inferior edge of the muscle, dividing into the anterior and posterior tibial arteries.

Probably the formation of aneurysm is accelerated in vessels that are atherosclerotic and are subjected to repeated trauma. In 1952 Palma,<sup>6</sup> in his study of stenosis and stenotic arteriopathy of Hunter's canal, suggested that pathologic changes occur because of repeated microtrauma secondary to systolic expansion of the vessel wall. Also, the effect of trauma to the popliteal artery during flexion and extension of the knee joint has been emphasized by Boyd and co-workers<sup>5</sup> and Lindblom.<sup>7</sup>

Observations in two recent cases having aneurysms of the proximal and distal popliteal artery and in another case having bilateral distal popliteal aneurysms lend support to the etiologic role of post-stenotic dilatation in formation of such aneurysms.

#### Illustrative Cases

##### Case 1

A 70-year-old man with diabetes mellitus was referred to the Mayo Clinic because of severe burning pain, numbness, and coldness of 9 days' duration in the right leg. He had been aware of claudication in both calves for about 1 year.

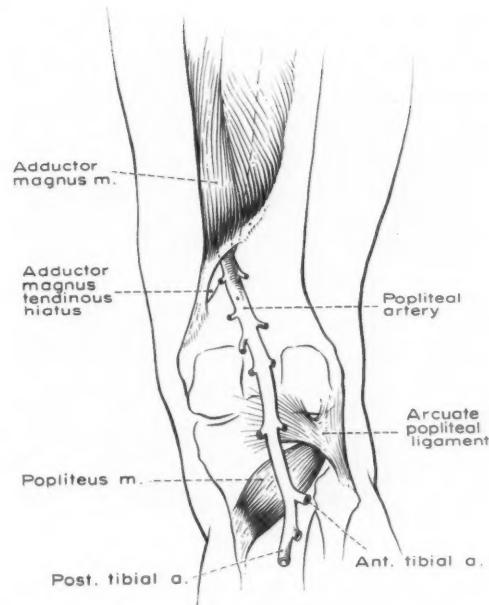


Figure 1

*Anatomic drawing of popliteal artery showing its relationship to arcuate popliteal ligament.*

On examination he was normotensive. He could not move the toes of the right foot, and they appeared mottled and cyanotic. The right anterior tibial muscle was edematous and moderately tender. The veins of the right leg were prominent. The dorsalis pedis and posterior tibial pulses were absent on the right, and a 6-cm. pulsating oblong mass was palpable in the right popliteal fossa. Prominent pulsations were present in the left popliteal fossa also.

The diagnosis was bilateral popliteal aneurysms and distal embolic occlusion from the right aneurysm.

Because gangrene developed in the right foot, a right midthigh amputation was necessary. Gross examination showed that the right femoral artery was enlarged to five times its normal size. The dissected specimen revealed two distinct popliteal aneurysms separated by a segment of relatively normal-appearing artery (fig. 2). The proximal aneurysm measured 7 by 6 by 5 cm. and was located immediately distal to the adductor magnus hiatus; the distal aneurysm measured 6 by 5 by 4 cm. and was located immediately distal to the arcuate popliteal ligament.

##### Case 2

A 59-year-old man was seen at the Mayo Clinic with the complaint that for 6 months walking

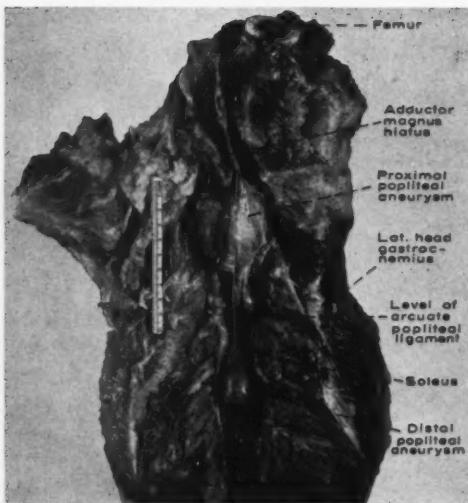


Figure 2

*Case 1. Photograph of popliteal fossa in dissected amputated limb of patient having proximal and distal popliteal aneurysms.*

one to two blocks had produced pain in the left calf.

On examination he was normotensive. Pulsations were absent from the left popliteal, posterior tibial, and dorsalis pedis arteries. On elevation of the left foot, pallor was moderately severe; and venous filling time was prolonged. There was no palpable aneurysm.

A left femoral percutaneous arteriogram showed no filling in a segment supplied by the distal femoral artery and proximal popliteal artery. There was good filling distal to this segment.

On surgical exploration of the left popliteal space, the popliteal artery was thrombosed distal to the adductor magnus hiatus. Between the heads of the gastrocnemius at the level of the arcuate popliteal ligament there was a narrowed segment in the popliteal artery with a small aneurysm distal to this point. A segment of popliteal artery was excised, and an alcohol block of the left lumbar sympathetic ganglion was performed.<sup>8</sup> Recovery was uneventful.

Four years later this man returned because of severe pain of 4 days' duration in his right calf. Since his last examination and surgery he had had claudication of both calves after walking three blocks rapidly.

Examination revealed exquisite tenderness, tenueness, and edema of the right calf with minimal edema of the ankle. There was moderate venous distention. In the left popliteal, dorsalis

pedis, and posterior tibial arteries pulsations were present but moderately diminished in amplitude. The right pedal pulses were difficult to assess because of edema, but the pulsation of the right popliteal artery was prominent low in the popliteal space and even in the upper part of the calf.

A diagnosis of acute right sural thrombophlebitis was made, and the possibility of its being secondary to a popliteal aneurysm was considered.

A right femoral arteriogram showed questionable evidence of a popliteal aneurysm. On surgical exploration, an aneurysm of the distal popliteal artery was palpable anterior to the medial head of the gastrocnemius. It measured approximately 6 cm. in length and 3 cm. in diameter. A right lumbar sympathectomy was first performed, and then the popliteal artery was cross-clamped and transected above and below the aneurysm. A Teflon graft was inserted. The patient's postoperative course was satisfactory.

#### Case 3

An 82-year-old man was referred to the Mayo Clinic because of pain, coldness, and numbness of 10 hours' duration in the right leg and foot. He was unable to move the toes of the right foot. He had known of the presence of an aneurysm of the right popliteal artery for 2 years, and for the same period he had experienced bilateral calf claudication on walking one half to one block.

On examination the blood pressure was 210 mm. of mercury systolic and 90 diastolic. The right leg was cold below the knee and the right foot was markedly pale. The patient was unable to move his right foot or toes. On the right, popliteal, dorsalis pedis, and posterior tibial pulsations were absent; and on the left, dorsalis pedis and posterior tibial pulsations were absent and popliteal pulsations were reduced. There was marked pallor on elevation of the right foot and moderate pallor on elevation of the left foot. Venous filling time was greater than 60 seconds on the right and was 30 seconds on the left.

Conservative treatment with a hot room, whiskey, anticoagulants, and papaverine resulted in improvement of the right foot with increase of warmth, sensation, and motor power over a 2-day period. The anterior tibial muscle became edematous, however, and the venous pattern of the right leg became more prominent. After 6 days the right lower leg became more edematous and the popliteal pulsations more diffuse. A leaking popliteal aneurysm or venous thrombosis was suspected.

Ultimately gangrene of the right foot and leg developed, and a midthigh amputation was performed. Dissection of the specimen disclosed two popliteal aneurysms. The first was just distal to

the adductor magnus hiatus, the second just distal to the arcuate popliteal ligament.

#### Summary

Two cases presenting complications of popliteal aneurysm requiring amputation have been presented. Dissection of these limbs showed proximal popliteal aneurysms located immediately beyond the adductor magnus tendinous hiatus and, in addition, distal popliteal aneurysms immediately below the arcuate popliteal ligament.

Another case is described in which bilateral popliteal aneurysms were located in the distal popliteal artery—that is, immediately below the arcuate popliteal ligament. These findings support the concept that post-stenotic dilatation may be one of the causal factors in the pathogenesis not only of proximal but also of distal popliteal aneurysms.

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Being venturesome involves taking risks. The risks will vary with different disciplines. Chemists have been killed or seriously maimed in their efforts to discover new kinds of explosives. Bacteriologists have been rendered desperately ill or have died from diseases for which they have been endeavoring to find a cure. Workers with the X-rays, in the early days before the dangers were realized, lost the use of fingers and hands or became horribly mutilated by the destructive energy of that powerful agent. In other realms of research the dangers may not be so serious, but all research is fairly certain to involve at least the regrettable risk of losing time. "Uncertainty and loss of time," as Emerson wrote, "are the nettles and tangling vines of the self-relying and the self-directed." Since time runs in only one direction, the eager investigator always looks upon its loss with sorrow. In my own experience I have too often had only labor for my pains. If the time I have spent in fruitless efforts to obtain control of the workings of the thyroid gland could be added to the end of my days, my span of life would be prolonged, I feel sure, by some years.—WALTER B. CANNON, M.D. *The Way of An Investigator*. New York, W. W. Norton & Company, Inc., 1945, p. 29.

# Heart Disease and Workmen's Compensation

## What Are the Costs to the Insurance Carrier?

By ROBERT D. RUSSELL, ED. D., AND RODNEY R. BEARD, M.D.

“EVERY CARDIAC INJURY CLAIM gets an award, and every award is a sizable one.” This generalization concerning the awards under the Workmen’s Compensation Act of the State of California stands as an increasingly obstinate barrier to the rehabilitation of cardiac cases in this State. The recognition of this fact was translated into a tangible form of action in 1955 when a team of research workers sponsored by the Cardiac in Industry Committee of the California Heart Association undertook a study of “Heart Disease Claims under the California Workmen’s Compensation Act”<sup>1</sup> — in order to substitute some facts for opinions in the crucial area of whether or not a cardiac accident is work connected. The authors concluded that heart disease claims filed in the study period (1948-1951) were not very great in comparison to the size of the State’s population and to the number of deaths caused by heart disease; heart claims constituted 1.7 per cent of all claims decided by the Industrial Accident Commission during this period. They noticed discrepancies in judgment among physicians and concluded that education of physicians for the part they play in these case proceedings is needed. Their final statement said, “It may also be pointed out that the allegation that ‘every heart claim gets an award’ has not been substantiated by this study.”<sup>1</sup>

In 1959 the present authors organized a follow-up study, sponsored by the Committee on Rehabilitation of the California Heart Association. This study was designed to at-

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This study was sponsored by the Rehabilitation Committee of the California Heart Association; this report is presented through the Committee.

tack the area of costs—what the insurance carriers (and, ultimately, the employers) paid to workers or survivors filing cardiac accident claims for the period 1948-1951. The Committee would like to offer these data in a comparative way with figures from other states and with cost data associated with other types of injury. Yet it seems that the information for comparison is not available; this investigation, then, must stand on its own or serve as a basis for later comparison.

The authors also are fully aware that costs for the period 1948-1951 cannot be accepted as directly representative of costs a decade later. The cases from this 4-year span were selected because they were the cases used in the original study and because during this span all cardiac cases went to a referee of the Industrial Accident Commission for disposition. In subsequent years all cases have not gone to referees, which introduces another variable into such a cost investigation. Thus it would seem that the figures for any time span—no matter how recent—would have to be qualified. The results of this investigation are offered as a point of departure and a base for further study.

A total of 523 case-record abstracts (filed in the Northern and the Southern California offices of the IAC) were available for use in the selection of a study sample. In the original study the cases for each district office were numbered consecutively; using a table of random numbers, the investigators selected 100 cases and 14 alternates to be included in the sample.

Out of the total of 114 cases, two were found to be noncardiac injuries and five (4 per cent) offered no cost information of any kind, leaving a working sample of 107 cases.

The procedure for gathering the data consisted of tracking each selected case back to

the insurance carrier and obtaining, either through a mailed form or the investigator's personal visit, all the cost data available. When the company was unable to provide information (due to destruction of records) the investigator went back to the case summary developed and utilized in the original Beard study, which provided the major cost items in most cases.

The information sought was as follows:

1. Amount paid on Temporary Disability (amount per week, number of weeks, and total amount).
2. Amount paid for Permanent Disability (amount per week, number of weeks, and total amount—for both Permanent Total Disability and Life Pension).
3. Amount paid for medical care.
4. Amount paid for Subsequent Injury.
5. Amount paid in Death Benefits, either as direct benefits or as burial expense.
6. Amount of Compromise and Release settlement.
7. Amount of other Direct Costs (investigations, phone charges, medical examinations, etc.).
8. Amount in reserve set aside if the injured is still living.
9. Amount held in reserve for future medical costs.
10. Amount of Indirect Costs.

Sixty of the cases (56 per cent) provided "full information," defined as those cases in which items 1 through 8 in the foregoing list were included or apparently nonexistent. Forty-seven cases (44 per cent) were represented by partially complete figures; in 38 of these (35 per cent of the total) the information came from the abstracted case record previously referred to.

The following profile of the sample can be sketched in from a review of the data\*:

\*A number of the case reports were much less precise as to the exact breakdown of the award than the categories set up for this study; as a consequence, the investigator had to make a number of judgments—with consistency as one of the major guideposts—as to how particular cases and amounts should be considered.

Out of 107 cases in the sample,

1. Seventy-nine (74 per cent) received awards of some magnitude, while 28 (26 per cent) were denied any compensation.
2. Nine cases (8 per cent) were awarded Temporary Total Disability, six (6 per cent) were awarded Permanent Disability, four (4 per cent) received both, five (5 per cent) were awarded some unknown combination (including death benefits), making a total of 24 (22 per cent) who received awards of disability payments.
3. The most prevalent type of compensation was the Compromise and Release Settlement, which went to 46 of the cases (43 per cent); this form of settlement was at least part of 58 per cent of all awards.
4. Twelve cases were awarded statutory death benefits (12 per cent).
5. Medical payments were awarded in 37 of the cases (35 per cent); in addition, nine (8 per cent) were reimbursed for medical examinations in conjunction with hearing proceedings or death. Of the former group 17 (16 per cent) were compensated for both disability and medical care, 11 (10 per cent) received medical reimbursement plus a compromise and release settlement, four (4 per cent) were awarded a combination of medical care, temporary disability, and compromise and release, three (3 per cent) received medical plus death benefits, and two (2 per cent) medical expenses alone.
6. Six cases from the total sample (5 per cent) are still open and being paid (8 to 13 years after injury).
7. Out of the 114 cases initially selected for review, two were noncardiac; of the remaining 112 heart cases, 49 (44 per cent) were dead at the time the Industrial Accident Commission decision was rendered (fig. 1).

Because of the division of the sample into

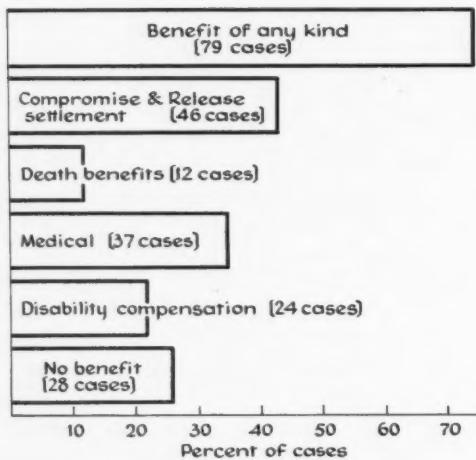


Figure 1

*Major varieties of Workmen's Compensation benefits awarded to a sample of 107 heart disease claimants, California, 1948-1951. (The percentages in this figure total more than 100 per cent due to the fact that five cases (5 per cent) received both temporary disability and a Compromise and Release settlement and thus were counted twice, and the fact that all but two (5 per cent) of those receiving medical treatment costs were awarded some other form of benefit as well.)*

those cases with reasonably complete information and those with partial or incomplete information the cost figures must show these two categories. Table 1 presents the mean and median total costs for a cardiac injury (rounded off to the nearest whole dollar).

If one considers total costs from a slightly different viewpoint, it can be shown that among the 60 cases for which data were complete, 20 (33 per cent) cost less than \$1,000, while 22 (37 per cent) amounted to \$6,000 or more. For 30 cases with incomplete figures on the payments paid, seven (15 per

cent) averaged less than \$1,000, and three (10 per cent) cost above \$6,000 each. Putting all the figures together, 27 (25 per cent) of the cases cost less than \$1,000, while 25 (23 per cent) received awards and incurred costs totaling \$6,000 or more per case (fig. 2).

There is a temptation to infer that the costs of the cases with "incomplete information" would approach those with "complete information" if all the data concerning them were available. This inference is probably incorrect. The major items of compensation payments and medical benefits were complete in most instances; the information lacking was mainly with respect to investigational costs, medical examinations, and similar relatively inexpensive items. Simply because they were more important, more complete records were kept on the high-cost cases.

The range in costs of the 79 cases receiving awards was from \$76.89 to \$30,541, with the latter case still "open" and receiving compensation. (Both of these were "complete" information cases.)

If one looks at the total cost picture in still another way, it can be shown that the six cases (6 per cent) with the highest awards (averaging just over \$15,000 each) cost the insurance carriers a total of \$90,610—or 26 per cent of the total awarded in cardiac cases for the sample in the 4-year period. To approximate this figure from the low compensation award cases would require 59 cases (55 per cent), the total costs from which total \$89,974 (26 per cent).

Total awards and costs have thus been presented in a number of ways; it would now seem appropriate to break these down and look at them in terms of the "categories"

Table 1  
*Mean and Median Total Costs for a Cardiac Injury, California, 1948-1951*

Mean total cost per case (full information).....	\$4,486
Mean total cost per case (incomplete information).....	1,584
Mean total cost per case (both).....	3,211
Median total cost per case (full information).....	3,291
Median total cost per case (incomplete information).....	713
Median total cost per case (both).....	1,663

spelled out in the earlier profile. Figures used in the succeeding paragraphs refer, then, only to that portion of the total sum paid which was designated temporary disability, medical, Compromise and Release, etc.

As was indicated earlier Compromise and Release settlements were the most frequent result of a cardiac injury claim. Of the 45 cases so settled the mean cost was \$2,427, the median cost was \$1,750, and the range was from \$9 to \$7,500, with 24 per cent of the cases under \$1,000 and only 15 per cent above \$4,000.

Nine cases from the sample (8 per cent) were awarded Temporary Disability. The mean cost of these awards was \$1,051, and the median cost was \$876.43. The range of judgments was from \$90 to \$2,790.

The six cases (6 per cent) receiving Permanent Disability represented a range between \$2,179 and \$9,496, with a mean cost of \$6,701, and a median of \$7,335. In these cases the awards tended to cluster in the \$6,000 and \$7,000 categories, so that disregarding the one low award would raise the mean to \$7,065. These figures can be considered indicative only of relative costs for a timespan, inasmuch as four of the six are still "open" cases. Two cases are receiving \$18.46 per week, one \$6 per week, and the fourth \$3.85 per week as a life pension.

A total of 24 cases (22 per cent) received disability awards of some type. The range of awards was from \$90 to \$30,541; the mean cost was \$5,431, and the median was \$4,649. As would be expected these disability cases were somewhat more expensive than the average; whereas the number of cases (24) represents only 22 per cent of the total working sample, the total cost of the cases represents 38 per cent of the total cost.

Twelve awards (11 per cent) were death benefits; the mean of these was \$5,471 and the median \$5,800. (Disregarding one very low award brings the mean figure to \$6,010, an even closer approximation to the median.)

Mean medical costs for the 37 cases in which this form of compensation was given were \$1,111, but this figure was greatly influ-

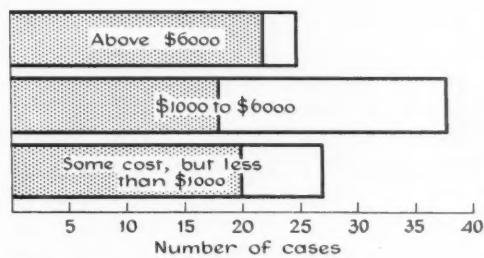


Figure 2

*Total costs per case of 90 cases of heart injury, California, 1948-1951. The 90 cases include the 79 that received awards plus 11 that received no award but cost the insurance carrier an identifiable sum of money. The 17 cases that cost nothing are not included. In these 11 cases, then, "received no award" is not synonymous with "cost nothing."*

enced by a few large awards. Specifically, it can be shown that the five cases receiving awards of more than \$2,000 (averaging just over \$4,000), while representing only 14 per cent of the total number, cost their carriers an amount equal to 52 per cent of the total cost of medical care. Without these five cases the mean cost is slightly more than half its original size (\$622) and more closely approximates the median expense figure of \$467. Four cases are still "open," the carrier being responsible for all further medical bills related to the cardiac condition.

The category "Other Direct Costs" contained a figure in 50 of the cases (47 per cent); the range of costs was \$2.50 to \$627.12, the mean cost \$142 and the median \$50. Again a rather large gap is noted between the mean and median, which is partially explained by the revelation that 50 per cent of the costs were under \$50 and a total of 64 per cent were under \$100, with 18 per cent between \$100 and \$200, 10 per cent between \$250 and \$350, and the final 8 per cent costing over \$350.

Another interesting group of figures available to the investigator involved time rather than money—and may be helpful in answering questions such as "how long does it take to complete a case?" or "How long is it, typically, between a cardiac injury and set-

tlement of the claim?" It was found that a mean period of 6.1 months passed between the day of injury and the day of filing the first claim with the Industrial Accident Commission (median—5 months). Nearly 50 per cent filed within 5 months, but 5.5 per cent took more than 2 years to take this action.

It also was found that the total mean time span from first Industrial Accident Commission entry until the last closing item was 11.4 months. If the three cases with long settlement periods (50 to 84 months) were left out, the mean dropped to 9.8 months, and if the mean were calculated without the top 10 per cent of cases it amounted to 7.5 months.

In answer to the question as to whether the two offices (Los Angeles and San Francisco) processed cases alike it was found that while the mean for San Francisco was 10.58 months and that for Los Angeles 12.83, the "t" test for the significance of difference between means showed a "t" value of 1.22, indicating that this difference readily could have occurred by chance.

In 40 cases the insurance carriers provided their closing dates, and for this group the mean time elapsing between injury and claim settlement was 39 months (median—32.5 months). About one third of the cases were settled in 20 months or less, and 12½ per cent took over 70 months; more than half fell into the category 10 to 50 months, or 1 to 4 years. (This does not include the six cases still open and being paid, where the mean time elapse since Industrial Accident Commission closure has been 9 years, 3½ months.)

In discussing the procedure utilized in this investigation the authors have concluded that whereas in some cases rather complete information finally was available from the insurance carriers, the number of these cases and the extent of the completeness did not justify such a time-consuming method. The investigator soon learned that private insurance companies make it a policy to destroy case records 5 to 10 years after the closure date. Undoubtedly the most efficient and

uniform procedure would be to use the total Industrial Accident Commission file as the source, compiling figures on awards only. (One of the results of this study, which showed that "Other Direct Costs" are reported as representing only 2 to 3 per cent of the total mean cost, would seem to justify this slightly more gross but greatly more efficient procedure.) Use of a common source, such as the Industrial Accident Commission file, would eliminate the variable of greatly varying precision in data reporting which must be admitted in preface to the conclusions of this study.

If one looks at the information presented as a basis for comment, it is interesting to note that most claims are settled rather quickly, with only 6 per cent still open (8 to 13 years after injury). (Two of these cases are receiving a life pension, two life pension and medical expenses, and two medical expenses only.) The majority of cases, then, are settled in less than 4 years.

Though this study does show again that every heart case does not get an award, it also discloses that slightly less than three-quarters of the claimants do get an award of some amount. In this regard, the fact that the insured died before settlement seems to be pertinent to the decision; 27 per cent of all cases in the sample received no award; 17 per cent of the deceased received no award, while 35 per cent of those surviving received nothing. (The treatment of these figures by the Chi square procedure shows the difference significant at the .03 level.) This would indicate that the survivors of deceased claimants were more likely to receive an award than would be the case if death had not occurred, chance being a very slight factor.

The report indicated earlier that the Compromise and Release settlement was the most common result of a case judgment. The reasons behind such an observed reality may be inferred from observations that follow:

1. Individual physicians may dispute one another's judgment in case testimonies. The Industrial Accident Commission considers

the judgment of any licensed physician to be as competent as any other. Where testimony is in conflict, the result is usually a Compromise and Release settlement. In general among the sampled cases, the indefinite statement of a single physician that the alleged injury "might have been work-connected" would change a "no award" case to a Compromise and Release settlement. However, in one instance a case in which all the facts seemed to point to a generous award was finally settled by Compromise and Release when one physician strongly averred that "there was no unusual strain to which this attack can be attributed . . ." 2. Claimants may be so bothersome that a carrier may compromise just to be rid of them. In one such case an award was denied 5 months after the claim was filed; 3 months later this denial was reaffirmed. One year later a case reopening plea was denied. Upon the injured's death (1 year later) the case was finally reopened, and a Compromise and Release figure of \$3,400 was agreed upon. The record states, "However, the defendants are willing to pay said sum to end litigation and buy their peace." 3. Some physicians may word their statements with sympathy for the patient as a guide. Douglass A. Campbell, J.D., the "dean" of the California Industrial Accident referees, stated, in an address to the California Heart Association ". . . that the accused event *might* have caused the heart attack is not scientifically sufficient for an opinion of causation . . . for every 'sympathy decision' . . . literally hundreds, if not thousands, of cardiac cripples will be denied the chance to work."<sup>2</sup> The Compromise and Release settlement, then, may symbolize both a lack of accurate, agreed-upon medical knowledge regarding heart disease and a social and economic value situation in which the desire that people should not have to be in need as a result of an injury is not yet matched by the proper structures to provide such funds.

If one assumes that the vast majority of cardiac accidents would require some amount of medical care, it is interesting to note that

only 35 per cent received such an award. Costs for this form of award seemed reasonable; the difference between mean and median figures is explained by the top four cases (11 per cent), whose awards averaged over \$4,500—including one case that accounted for 24 per cent of the total medical care awards. Thus, while the chance of high medical bills is present, it seems to be just about 1 in 10.

The total cost of a case can be expected to fall within the range of \$2,000 to \$4,500. The \$1,500 discrepancy between the mean and median figures emphasizes the importance of those who receive sizable awards (the 7 per cent of the claimants with the highest awards received monies equal to those received by 67 per cent who rated small awards). The same generalization would hold for Compromise and Release settlements, though the discrepancy difference here is less than \$700.

As expected, disability awards amounted to more than Compromise and Release settlements; roughly two-and-a-half times more.

In summary and conclusion, this report has provided some definite cost figures taken from a randomly selected sample of cardiac cases whose claims were heard by a referee of the California Industrial Accident Commission during the 1948-1951 period.

It shows that Workmen's Compensation benefits cost, on the average, about \$3,000 per case. Disability and death benefit awards accounted for 34 per cent of the awards and averaged just under \$5,500 each. Medical awards were made in 35 per cent of the cases and averaged \$1,111, though this figure was greatly influenced by a few large payments. The Compromise and Release settlement was the form of compensation in 43 per cent of the cases—and was at least a part of 59 per cent of the awards; mean cost of these cases was \$2,427. Only 6 per cent of the total sample were "open" cases (still being paid).

It further shows that about 6 months, on the average, elapsed before a worker filed a claim, and that the disposition required just less than 1 year. The mean time for completion of an award case with the insurance carrier was just over 3 years.

While reliable, comparable figures on the costs of Workmen's Compensation benefits for other disease or injury categories or for other States have not been available, we have the impression that the average in other cases in California is considerably lower, probably less than \$1,000. Thus, the occurrence of a compensable heart injury in a small business could lead to a perceptible increase in insurance premiums. However, as was previously shown, the probability of such happening is not great, due, largely, to the general policy of basing rate changes upon a much broader category of business than the unfortunate experience of a single small firm. Also, the majority of such cases occur as a result of arteriosclerotic heart disease, and most of them are in persons without previous knowledge of heart disease. Arbitrary exclusion from employment on the basis of having had "a heart attack" or "high blood pressure" or an abnormal electrocardiogram will not effectively conserve the em-

ployer's Workmen's Compensation insurance premiums, while it does unnecessarily blight the lives of many able people whose coronary artery disease has become evident. The answer to the problem of Workmen's Compensation costs lies in the appropriate work assignment of workers with heart disease (and all other workers) and the development of other forms of sickness and disability insurance which will make it less necessary to look to Workmen's Compensation as a source of support for the disabled, widowed, and orphaned.

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#### Religio Medici

Certainly that man were greedy of Life, who should desire to live when all the world were at an end; and he must needs be very impatient, who would repine at death in the society of all things that suffer under it.—SIR THOMAS BROWNE. *Religio Medici*, 1642  
Edited by W. A. Greenhill, M.D., Oxon., London, MacMillan and Co., Limited, 1950, p. 3.

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## SPECIAL ARTICLE

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### Reactivity of Cardiac Vessels and Reparative Processes Following Cardiac Infarction

By SERGEY V. ANDREEV, M.D.

**A**MONG VARIOUS FACTORS regulating the blood supply of tissues, the essential role belongs to the reactivity of blood vessels. Inadequate vascular reactivity, which is rarely observed in physiologic conditions, may occur rather frequently in pathologic situations. Inadequate and especially a distorted vascular reaction increases the circulatory derangement resulting in the delay of reparative processes. The changes in vascular reactivity are of great significance, mainly for the functioning of the heart.

In accordance with the above factors, there appears a very important assignment for the experimental investigations in the field of cardiovascular pathology, namely, for the studies of vascular reactivity in the heart and of different methods, which may lead to the activation of reparative processes following cardiac infarctions as well as of other organic impairments of the heart, which might become useful for the cardiologic clinic.

#### Materials and Methods

Vasomotor activity of the heart was studied with the aid of the motion picture, coronary onkography, and automatic registration of the amounts of nutrient fluid outflowing from the vessels of isolated heart.

A motion picture of the cardiac vessels was taken at the rate of 24 pictures per second in thoracotomized dogs exposed to the acute experiment and maintained under artificial respiration for a period ranging from 20 to 90 minutes. Films of the motion picture had registered the changes in form and size of the vessel lumina, occurring in

the time interval of 0.04 second. Observations were made before and after the intravenous administration of various vascular substances; two drops of a 1 per cent solution of nitroglycerin were applied to the dog's tongue, while the physiologic saline was substituted in the control experiments. After completion of the acute experiment the motion picture films revealing changes in blood vessels were studied with the aid of a photomagnifier.

A special onkograph made of plastics or of thin aluminum measuring 0.6 by 1.0 to 1.0 by 1.5 cm. was arranged behind the middle third of the descending branch of the left coronary artery where its posterior surface is almost free of vascular outbranchings. The onkograph was connected by means of a rubber tube with a special recorder, which transferred all the alterations of nutrient fluid on kymograph.

Comparative onkography was performed in coronary, femoral, renal, and carotid arteries.

Control records of vascular oscillations in carotid, renal, and femoral arteries were made in dogs with normal respiration (without opening of the chest) following intravenous administration of physiologic saline instead of various vascular agents.

The hearts of adult human beings were also under observation. In one of the four experimental series we have studied the hearts of persons who died from a severe injury. Histologic investigations showed that in approximately one half of all these cases the coronary arteries revealed no alterations, whereas in the remaining half the coronary vessels were atherosclerotic. The second series of such experiments was conducted on the hearts of individuals who died from acute coronary insufficiency (stenocardia), and the third series on hearts with cardiac infarctions. The fourth series was done on hearts of human embryos and children of different age groups.

Two series of experiments were made on dogs: in animals with normal myocardium and in those

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with experimentally produced cardiac infarction, which was induced by ligation of the descending branch of the left coronary artery. The reactivity of the cardiac vessels in the second series of experiments on dogs was studied in different stages of developing cardiac infarction.

The pressure and temperature of a nutrient solution flowing through aortic cannula into the cardiac vessels remained constant during the entire experiment; therefore, the alterations in coronary outflow have been considered as an expression of oscillations taking place in vascular lumina and, consequently, in vascular tone.

Calculation of the nutrient fluid passing through the cardiac vessels was accomplished automatically by special equipment resembling that of Condon.<sup>1</sup> The results of measurements were recorded on diagrams as curves showing the value of coronary outflow in milliliters per minute.

Bioelectric potentials from the superior vena cava of a man and from the heart muscles of a man and a dog were lead out through unpolarizing brush electrodes and were recorded in the stationary or portable electrocardiograph.

The dynamics of bioelectric potentials in the heart muscle were studied after the introduction of various vascular substances in the dog's body and in isolated human and animal hearts. The solutions of vascular agents were administered in the following concentrations: nitroglycerin, 1:210<sup>2</sup> to 1:3.10<sup>3</sup> euphyllin, 1:5.10<sup>3</sup> to 1:2.10<sup>3</sup> k-strophantin, 1:8.10<sup>4</sup> to 1:10<sup>4</sup> adonizide and tincture of digitalis, 1:210<sup>2</sup> to 1:10<sup>2</sup>.

Activation of reparative processes in the heart muscle was studied in 129 adult albino rats and 109 rabbits. In the region of the middle third of the anterior ventricle wall, injury to myocardium was applied in all rats by a specially arranged eye-pincer. The area of the injured myocardial surface was 1.5 to 2.0 mm.<sup>2</sup>; its depth was 1.0 to 1.5 mm.

In 71 of the rabbits trauma to the heart muscle was also applied by means of the modified Kocher's pincer. The injured area was 6 to 10 mm.<sup>2</sup>; its depth, 1.5 to 2 mm. In the remaining 38 rabbits, experimental cardiac infarction was produced by ligation of the descending branch of the left coronary artery in its upper third.

For evaluation of the functional state of myocardium the bioelectric potentials were studied in all animals as well as the histologic pattern of myocardial changes. One half of all the rats received vitamins and aminoacids by subcutaneous injections, once every 2 days in the following doses: cobalamine, 10 to 30 µg.; methionine, 15 to 30 µg.; adenosinetriphosphate, 1.0 to 3.0 mg.; deoxyribose nucleic acid, 10 to 30 mg.; pyridoxine, 50 to 150 µg.; triptophane, 1.0 to 2.5 mg.

### Results

Our observations have demonstrated that the tone of cardiac vessels may change in the form of rapid and, to some extent, independent, rhythmic oscillations or constrictions of the vascular wall. Simultaneously with frequent rhythmic constrictions of the vascular wall there is a manifestation of protracted changes in the general vascular tone, which may result in either contraction or dilatation. Both types of vasomotor activity may alter under the influence of various vascular substances. Depending on the type of vasomotor response, namely, whether it will be adequate or inadequate to the introduction of certain vascular substances, it is, to some extent, possible to characterize the functional state of vascular tone — its reactivity.

Independency of the rhythmic constrictions of the vascular wall is confirmed by the following factors: 1. During the restoration of cardiac activity there appears an intense and protracted (up to 2.5 hours) constriction of the superior vena cava in the human cadaver long before the onset of any contractile activity of the heart. Some time later the records of bioelectric potentials in human superior vena cava show arrhythmic constrictions (144 to 300 per minute) also in relation to the contractions of the heart. 2. As the cardiac function in man restores, the vasomotor activity in the heart may appear in the absence of and independently of myocardial contractions. 3. An amplitude of arterial oscillations does not always reflect the intensity of cardiac contractions and alterations in the level of the entire arterial pressure. 4. The form of oscillations in individual arteries may change independently of each other (for example, under the influence of caffeine, epinephrine, and amylnitrite).<sup>2, 5</sup> 5. Periodic changes in width and length of cardiac arteries and arterioles, small veins and venules take place in time intervals of 0.04-0.06 to 0.8-0.12 second. These time intervals do not always coincide with the contractions of the heart muscle.<sup>6</sup> The peculiarities of vasomotor activity are characterized by the three types of rhythmic constrictions of vessels, which

differ from one another in amplitude, form, and frequency. Alterations of vascular tone are significantly obvious in type I and mildly obvious in type III. Type II occupies an intermediate position (fig. 1).

Arterial oscillations have a definite significance in the blood supply of the heart muscle. In type I, with a relatively greater amplitude of oscillations, the cardiac vessels are able to pass through themselves approximately twice as much nutrient fluid as in type III ( $224 \pm 12$  ml. and  $109 \pm 5$  ml. respectively) and in type II ( $161 \pm 8$ ). Contractile function of the human heart muscle may be restored after death more frequently (in 45 cases of a total of 54) during the intense (types I and II) vasomotor activity.<sup>3, 4, 7, 8</sup>

In some diseases and intoxications of the body (toxic dysentery, diphtheria, bronchopneumonia, acute stage of cardiac infarction) the vasomotor activity of the cardiac vessels is strikingly decreased and is very close to complete exhaustion. However, it increases in its intensity and appears as a compensatory factor, which enhances the blood supply of the heart in angina pectoris, in severe atherosclerosis, in hypertensive disease, and in the acute and subacute stages of cardiac infarction in man and in experimentally produced cardiac infarction in dogs.

Caffeine, amylnitrite, nitroglycerin, adonizide, sodium nitrite, increpane, and especially strophanthin increase oscillations of cardiac vessels, whereas euphyllin decreases them. Epinephrine, against the background of a general elevation of arterial pressure, leads to a temporary decrease of oscillations, which remain on low levels for a few minutes.

Epinephrine inhibits the oscillations of the cardiac vessels, simultaneously increasing them in the peripheral arteries. This factor has to be taken into consideration for the evaluation of the role played by emotions in the blood supply of the myocardium, when epinephrine is discharged in increased amounts into the vascular bed.

The temperature of blood or of nutrient fluid averaging  $+37.0 \pm 1.0$  and the pres-

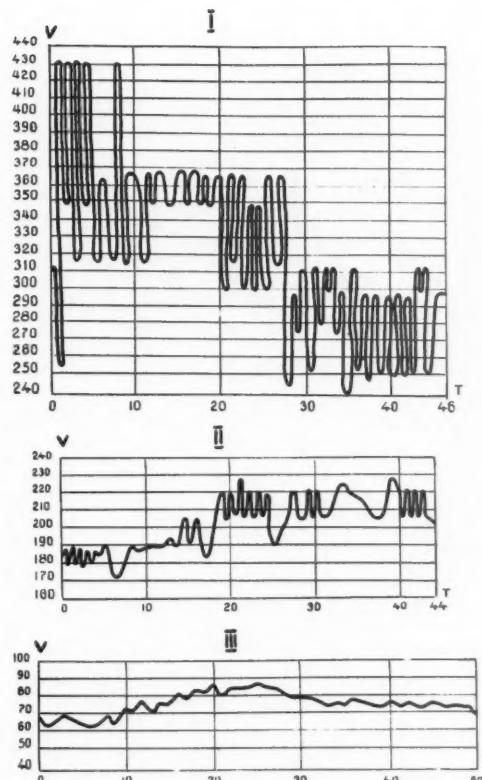


Figure 1

The curves of rhythmic vascular constrictions in various types of cardiac vessels: I, experiment 183; II, experiment 129; III, experiment 73. Vertical axis corresponds to the volume of nutrient medium passing through cardiac vessels (V) in milliliters; horizontal axis (T) in minutes.

sure limited to  $80 \pm 20$  mm. Hg is considered optimal for arterial oscillations. In some experiments human heart vessels have revealed a quality of changing their tone quite rapidly (in a period of 1 to 3 minutes) in such a way that coronary outflow has been altered from two to sevenfold volume (from 155 to 305 ml. and from 415 to 53 ml.) (fig. 2).

Dynamics of changes in vascular tone of the human heart may have different significance: an adaptive, which tends to increase the blood supply of the heart muscle and is antagonistic, which induces hypoxia, and ischemia of the heart muscle leading to cardiac necroses.<sup>4</sup>

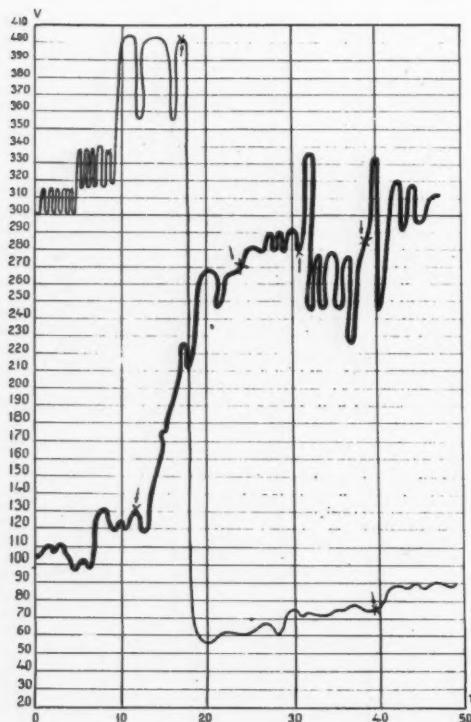


Figure 2

Rapid changes in the tone of cardiac vessels (designations on vertical and horizontal axes are the same as in figure 1). Thin, almost vertical, line indicates the rapid rise of vascular tone observed in experiment 134, under the influence of euphyllin solution (1:5.10).<sup>3</sup> Thick line indicates the diminution of vascular tone following administration of nitroglycerin solution (1:3.10)<sup>3</sup> in experiment 123.

Change of elevation of the vascular tone by its diminution, which may occur as a two-phased reaction (under the influence of intravenous administration of epinephrine) is sometimes observed in intact, healthy animals.<sup>2, 6, 10, 11, 12</sup> Sometimes, in the physiologic condition, a two-phased vascular reaction may occur after introduction of several other substances (nitroglycerin, atropine, histamine, inerepane). The two-phased or distorted reaction of cardiac vessels in man was encountered in the acute stage of cardiac infarction, in stenocardia, in atherosclerosis, in hypertensive disease, and in the condition

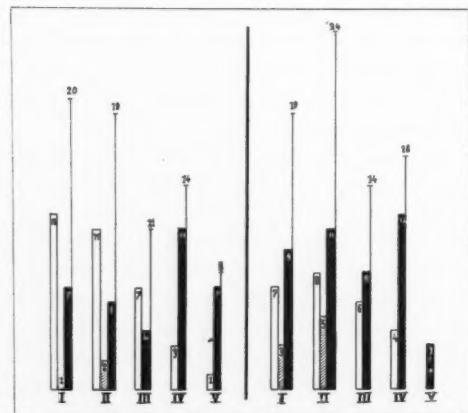


Figure 3

The effect of pharmacologic substances upon the human cardiac vessels following mechanical injury: left, upon the intact coronary vessels; right, upon the coronary vessels impaired with atherosclerosis. I, nitroglycerin; II, ephedrine; III, strophanthidin; IV, adoniside; V, tincture of digitalis. Vertical thin lines designate the total number of observations; black columns, spasmodic vascular response; white columns, vascular dilatation; column with oblique lines, the change of first spasmodic phase into vascular dilatation. Arabic figures within columns indicate the number of experiments.

following craniocerebral injury and injury of somatic organs, which are followed by manifestations of traumatic shock, and in the first period of experiments with the restoration of human heart activity after death, when metabolic processes in heart muscle, according to the data of spectrographic investigations, were obviously impaired.<sup>13, 14</sup>

In the acute stage of cardiac infarction the vasodilating effect of nitroglycerin was observed in only one of eight experiments; in the remaining seven a two-phased or spasmodic reaction was present (fig. 3).

Human cardiac vessels being free of organic changes had shown in the majority of experiments an ability of dilating under the influence of ephedrine and nitroglycerin. Nitroglycerin and strophanthidin caused more manifested dilatation of cardiac vessels, which were affected by atherosclerosis in comparison with the intact coronary arteries; ephed-

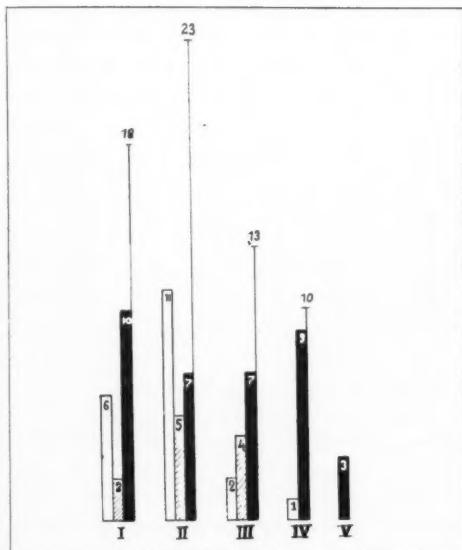


Figure 4

*The effect of pharmacologic substances upon the human cardiac vessels following angina pectoris. Designations are the same as in figure 3.*

lin, on the other hand, caused more intensive dilatation of the intact vessels.

Introduction of adoniside and digitalis had resulted in more intense constriction of atherosclerotically changed cardiac vessels in comparison with the intact ones. It is therefore evident that atherosclerotically altered coronaries of a man possess more manifested reactivity to vasodilating and vasoconstricting agents.

The tendency of the heart vessels to spasmodic reactions, despite the effect of euphyllin, was more frequently observed in conditions following craniocerebral injury, traumatic shock, or after derangement in brain circulation (insult) appearing against the background of hypertensive disease. The same tendency had been demonstrated in approximately half of the experiments following hypertension (fig. 4).

The most constant spasmolytic effect upon the vessels of human heart in the acute stage of cardiac infarction and in conditions following acute coronary insufficiency (stenocardia) was observed after administration of

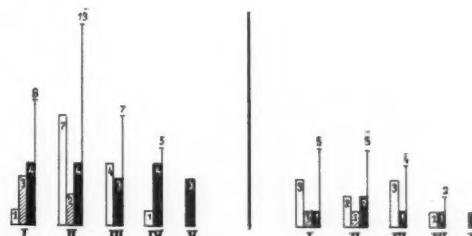


Figure 5

*The effect of pharmacologic substances upon the human cardiac vessels following cardiac infarction: left, the acute state; right, the subacute stage. Designations the same as in figure 3.*

euphyllin and strophanthin. Euphyllin possesses this quality even in the cases in which nitroglycerin causes spasmodic vascular reaction (fig. 5).

Spasmolytic effect was noted after administration of low concentration of euphyllin, nitroglycerin, and strophanthin, whereas high concentrations of these pharmacologic agents causes spasmodic reactions and seemed to be inadequate to the functional state of heart vessels in man during the acute stage of cardiac infarction and in conditions following acute coronary insufficiency. This phenomenon was also encountered in the acute stage of experimentally developing infarction in dogs.

In the acute stage of experimental infarction a spasmolytic effect has been revealed only in some individual cases, whereas in the remaining ones the spasmodic vascular reaction had predominated in the coronary arteries of dogs. Inadequacy of the vascular reaction in experimental cardiac infarction was demonstrated in the isolated heart as well as in the entire organism during investigations of bioelectric activity of the heart. (fig. 6).

Experiments on dogs have confirmed our observations concerning inadequate vascular response to the introduction of some vascular agents in the course of cardiac infarction in man.

Negative influence of focal impairments in the cardiac muscle upon the entire body and upon the reactivity of cardiac vessels

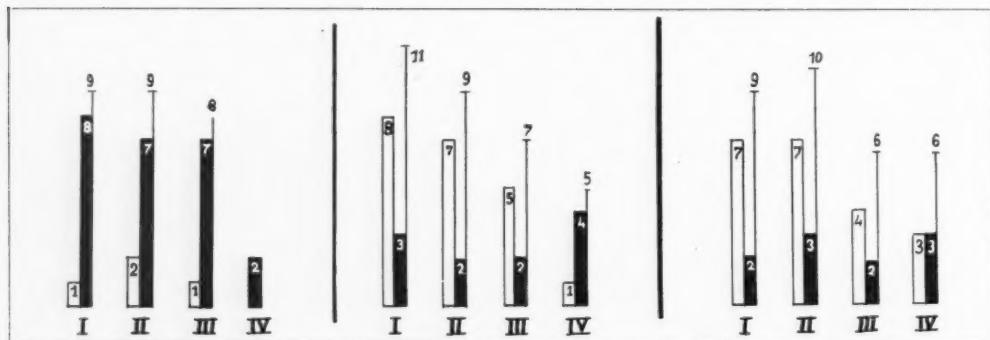


Figure 6

*The effect of pharmacologic substances upon the cardiac vessels of dogs with experimental cardiac infarction and of control animals: left, reaction in the acute stage; center, reaction in the subacute stage; right, reaction in the intact dogs. Designations are the same as figure 3.*

brought about the necessity of experimental studies on the problems associated with the activation of reparative processes in the cardiac muscle.

The combination of vitamins used in our studies (cobalamin, pridoxine) and amino-acids (methionine, desoxyribonucleic acid, adenosinetriphosphate, and triptophane) was considered as a measure directed to the intensification of processes concerned with biosynthesis of proteins in the body, activation of transamination, transmethylation, phosphorylation, and transsulfuration. Thus, we intended to improve the reparative processes in the area of focal derangements and necroses in the heart.

The best results were obtained in the series of experiments on rats (with injury of the myocardium) and on rabbits (with cardiac infarctions) that received a complex treatment with cobalamin, methionine, adenosinetriphosphates, and desoxyribose nucleic acid. Normalization of the electrocardiogram in the hearts of animals so treated seemed to be faster than in the control animals (sixth to twenty-eighth day and fourteenth to forty-eighth day in rats and sixth to thirtieth and tenth to sixtieth day in rabbits, respectively).

In animals receiving the above treatment the disappearance of necrotic muscle fibers and the production of the mature connective

tissue took place in a significantly shorter period. The development of connective tissue in the majority of these cases was limited to the areas of injury; furthermore, the nerves in such areas as well as in the regions of scar formation, showed no significant changes.

A complete healing of the cardiac muscle in control rats took place on the thirtieth to the fortieth day; in control rabbits, on the thirtieth to the sixtieth day, whereas in animals receiving complex treatment this happened much earlier (on the fifteenth to the twenty-fifth day in rats and on the twenty-fifth to the fortieth day in rabbits).<sup>8</sup>

Consequently, the treatment that we used (a combination of vitamins with amino-acids) in rats and rabbits having focal lesions of the heart showed a positive effect and had accelerated the reparative processes in the heart. This may have a practical significance in the clinic of internal medicine.

#### Discussion

Exclusively complex neurohumeral regulation of hemodynamics in man and in animals only allows to outline some of the mechanisms in the regulation of vascular tone.

I. P. Pavlov, studying neural mechanisms in the regulation of the vascular system, had stated that a significant role in this process

belongs to the chemical substances that are produced in the body. One of the principal problems of numerous investigators is to disclose the chief chemical processes that are essential in the impairments of vascular tone. Why does the vasomotor activity increase or decrease or, in some instances, completely disappear? Why do the changes in vascular tone lose their natural dynamics (replacement of elevating phase by the diminishing one) and become less mobile for a long time or reveal a tendency to spasmotic reactions?

One of the numerous causes leading to derangements in dynamics of vascular tone is hypercholesterolemia and an increased synthesis of cholesterol and, apparently, of other vascular substances (epinephrine, norepinephrine) in the vascular wall. This was demonstrated recently with great conviction by many authors, who in their investigations used radioactive isotopes.<sup>15, 18</sup>

Cholesterol enhances the tone of blood vessels, especially of arteries, and thus facilitates their transition into the spasmotic state. The impairment of phosphorylation, conditioned by deficiency of adenosinetriphosphate, which is characteristic of lesions appearing in the heart, may augment the sensitivity of vessels to spasmotic agents. In experiments with atherosclerosis the vessels of the rabbit's ear respond more intensely to the vascular substances and reveal a tendency to spasmotic reactions.

Hypercholesterolemia appears in a human body as a consequence of focal disturbances in the brain, hypertensive disease, atherosclerosis, intoxications, and avitaminosis. In the diseases mentioned above derangements in vascular tone were manifested by inadequate or distorted vascular reaction. The significance of the cholesterol dynamics in the blood for the reactivity of blood vessels confirmed by the fact that some substances (particularly, sodium fluoride), which reduces the amounts of cholesterol in the blood and causes the lowering of blood pressure, augments the effect of nitroglycerin upon the cardiovascular system during chronic experi-

ments on animals; thus, the adequate reaction of vessels to the effect of nitroglycerin may be increased.

In atherosclerosis the synthesis of phospholipids and of cholesterol in the walls of the blood vessels significantly increases. Deficiency of pyridoxin and choline in the body may cause lipomatose infiltration of vessels.

When vascular tone is impaired (in hypotension) the content of thiamine in the blood falls significantly as well as that of ascorbic acid and carotin, although in the latter to a less extent. Deficiency of thiamine in the body predisposes the tissues of heart muscle and of blood vessels to the development of focal necroses.

The possibility of mutual transformation of cholesterol into sugar in the body is one of the indicators of close relations between the disturbances in cholesterol and carbohydrate metabolism. These predispositions are enhanced by the facts that a great number of patients with diabetes mellitus suffer from angina pectoris and that diabetic acidosis is often accompanied by the development of focal necroses in the myocardium.

Our knowledge of vascular tone is becoming more concrete. Vasomotor activity dependent upon the central nervous regulation and upon the function of numerous angioreceptors, which are connected directly with the heart muscle by nerves,<sup>19</sup> could be as well defined by very complex synthetic processes taking place in the walls of the vessels and resulting in the production of various vasoactive substances.

Intoxication due to dysentery, diphtheria, or pneumococcal infection suppresses the vasomotor activity as a result of disturbances in general metabolism and, possibly, in the walls of blood vessels simultaneously with the functional and structural alterations in angioreceptors. Focal derangements of vessels and of brain tissue in intracerebral hemorrhages, chronic irritation of the descending branch of the left coronary artery by a ligature in animals, cardiac infarctions in man and in experiments, and pathologic and experimental constriction of renal arteries

are followed by impairment of vascular tone (in a form of hypertension or hypotension) and of reactivity in the blood vessels up to complete distortion of vascular reaction. The reduction of vasomotor activity and distortion of vascular reaction may be encountered most often in an acute period of focal lesions; for instance, in the acute stage of cardiac infarction, when coronary blood supply may be diminished to 30 per cent. Focal lesions of the heart muscle also cause transformation of its vascular network.<sup>20, 21</sup>

Reflex influences, arising in the lesion and general toxic processes alter the metabolism in vascular walls provoking profound and protracted changes in vascular tone, which may be revealed in a form of hypertension or distorted reactivity of vessels.

Our experiments have demonstrated that the genesis of spasmody reaction of the cardiac vessels may be determined, not only by the atherosclerotic process, but also by a neurohumoral influence of necrotic focus upon the metabolism in the heart muscle and in the vascular walls as well.

Morphologic studies reveal zones of ischemia (arteriolospasm) around the necrotic focuses in the myocardium, e.g., the two-phased changes of vascular tone. Thus, functional and morphologic studies reveal the role of necrosis in the derangements of vascular tone. Atherosclerotic disturbances of metabolism in the vessels are accompanied by the augmentation of their reactivity, due to vascular agents, especially of the spasmody type.

Besides the above-mentioned factors of metabolism (thiamine, pantothenic acid, adenosinetriphosphate, cholesterol, insulin, increpan, epinephrine, magnesium, and potassium) other factors no doubt participate in the processes of regulation of vascular tone.

Focal lesions of the heart muscle, which cause profound derangements in metabolism of the heart, as well as of the entire body, require the application of adequate and complex treatment having a multilateral effect.

The complex treatment, which we used to accelerate the reparative processes in the

heart muscle, is only a starting point in our studies in this direction.

#### Summary

It was stated that the human heart possesses a high vasomotor activity: in 1 to 3 minutes the coronary vessels may pass into a state of spasm or dilatation in such a manner that the volume flow may change up to 2 to 7 times. Apparently, this phenomenon discloses one of the mechanisms leading to prolonged spasm of the coronary arteries.

Vasomotor activity of the heart is characterized by three types of rhythmic oscillations of vessels, which differ from one another in amplitude, form, and frequency. In some intoxications (dysentery, diphtheria, pneumococcal infection) and diseases, vasomotor activity tends to decrease, whereas in the subacute stage of cardiac infarction in man and in dog, in angina pectoris, in atherosclerosis of the coronary vessels, and in hypertensive disease, it seems to be compensatorily augmented.

Two-phased or distorted reaction of heart vessels is determined not only by the atherosclerotic process but by neurohumoral influences of necrotic focus upon the metabolism in the heart and in the vascular wall.

The most constant spasmolytic effect upon the vessels of the human heart was obtained by the administration of euphyllin and strophanthin in low concentrations.

It was demonstrated that, in addition to vitamins, hormones, aminoacids, and mineral salts, many other factors participate in the regulation of vascular tone.

Acceleration of reparative processes in experimentally produced focal lesions of the heart was encountered after application of a complex treatment on animals with vitamins and aminoacids (B<sub>12</sub>, adenosinetriphosphate, methionine, and desoxyribonucleic acid).

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In the treatment of disease it was with Sydenham a cardinal principle to interfere as little as possible with the *vis medicatrix naturae*. He adopted as his own the fundamental concepts of Hippocrates, "Nature is the healer of diseases."—DAVID RIESMAN, M.D. *Thomas Sydenham, Clinician*, New York, Paul B. Hoeber, Inc., 1926, p. 36.

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## CLINICAL PROGRESS

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### Surgical Treatment of Dissecting Aneurysm of the Aorta Analysis of Seventy-Two Cases

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DENTON A. COOLEY, M.D., E. STANLEY CRAWFORD, M.D.,  
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**D**ISSECTING ANEURYSM of the aorta is a serious condition, which until recently has proved fatal in more than 75 per cent of cases.<sup>1-4</sup> The disease was clearly recorded by Morgagni<sup>5</sup> as early as 1761, and Laennec<sup>6</sup> in 1819 designated it as "aneurysme dissequant." In spite of this early recognition and the subsequent increasing interest in the condition, the natural course of the disease remained unaltered until about 6 years ago, when methods of surgical treatment were devised.<sup>7</sup>

Spontaneous healing of a dissecting aneurysm has been recognized for more than a century. Shekelton<sup>8</sup> of Dublin, in 1822, and Henderson<sup>9</sup> of Edinburgh, in 1843, reported cases in which the aneurysms ruptured into the lumen of the aorta or an iliac artery at some distal point. Endothelial growth at the sites of rupture was so complete in these "healed" cases that Hope<sup>10</sup> referred to the double aorta as a congenital anomaly. Even though this method of nature to promote healing was apparently well recognized, it was not until 1935 that Gurin, Bulmer, and Derby<sup>11</sup> utilized it to treat a patient with right iliac

block produced by dissection. They were able to restore circulation to the extremity by creating a re-entry passage at the site of the iliac obstruction, but death resulted from renal failure. In 1948 Paullin and James<sup>12</sup> attempted to strengthen the wall by wrapping cellophane about the dissected aorta, but the method proved unsatisfactory. Five years later, Johns<sup>13</sup> reported suture repair of a rather unusual form of ruptured dissecting abdominal aneurysm, but the patient died of renal failure. In 1955 Shaw<sup>14</sup> reported a typical case of dissecting aneurysm associated with acute arterial insufficiency of the lower extremities in which aortic obstruction was relieved by a procedure somewhat similar to that employed by Gurin and associates,<sup>11</sup> consisting essentially of an abdominal aortic fenestration with repair of the dissection distally, but the patient also died from renal failure.

Since our first successful operation approximately 6 years ago, we have treated 72 patients with dissecting aneurysms of the aorta. Although the basic principles underlying the surgical procedures employed in these cases are essentially similar, certain variations have been utilized, depending upon the location and extent of the lesion. Some form of excisional therapy with replacement by homograft or prosthesis has been the most frequent procedure. This report is concerned with certain significant observations derived from analysis of this experience.

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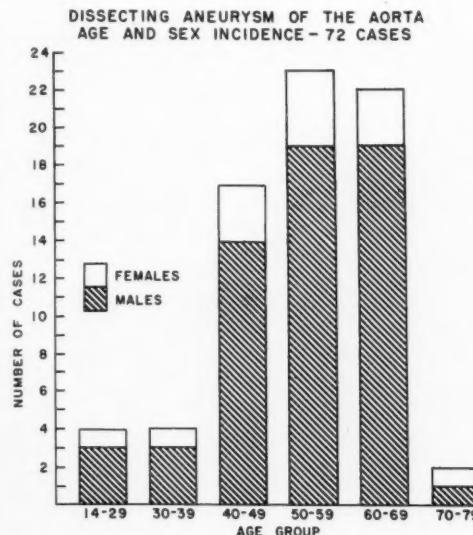


Figure 1

Distribution according to age and sex of 72 cases of dissecting aneurysms of the aorta.

### Incidence

Dissecting aneurysm of the aorta has been encountered in from 0.1 to 4.0 per cent of postmortem cases<sup>2, 4, 15-20</sup> comprising from 12 to 25 per cent of all aneurysms of the aorta.<sup>15, 18, 19, 21, 22</sup> Men are affected two or three times as often as women except in persons older than 80 years of age when this relationship is reversed.<sup>2, 23</sup>

In our experience with 1,281 cases of aneurysm of the aorta treated surgically, dissecting aneurysm comprised 6.0 per cent of the total number (table 1) and 20 per cent of the thoracic aneurysms. Of these 72 patients 88 per cent were men, and approximately 90 per cent were in the fifth, sixth, or seventh decade of life (fig. 1). Seven patients were younger than 40 years of age, and two were older than 70. Six of the younger patients had definite characteristics of Marfan's disease. The age range was from 14 to 74 years, with an average age of 54 years.

Although no age group is exempt from this condition, the highest peak reported by most authors has been between the fourth and seventh decades. Commenting on dissecting

Table 1  
*Incidence of Dissecting Aneurysms among All Surgically Treated Aortic Aneurysms*

Location	No. cases	Per cent
Aortic arch	60	5
Descending thoracic	134	10
Dissecting aneurysm	72	6
Thoracoabdominal	29	2
Abdominal	986	77
Total	1281	100

Table 2  
*Dissecting Aneurysm of the Aorta. Relative Frequency of Symptoms—72 Cases*

Symptom	No. of patients	Symptom	No. of patients
Substernal or precordial pain	48	Neurologic manifestations	7
Only symptom	6	Hemoptysis	2
Back pain	35	Impaired leg circulation	2
Only symptom	1		
Epigastric or abdominal pain	20	Hematuria	1
Only symptom	4	Shock	1
Dyspnea	11	Asymptomatic	2

aneurysms encountered in young persons, Schnitker and Bayer<sup>24</sup> reported almost 25 per cent of 580 cases in patients younger than 40 years of age, and 38 per cent of Gore's<sup>25</sup> cases were in this group. The high incidence of young individuals in these series is undoubtedly due to the selective nature of cases studied. A more likely incidence of 15 per cent of 505 cases has been reported by Hirst and co-workers.<sup>23</sup>

### Clinical Manifestations

Several recent reports have emphasized the characteristic clinical manifestations of this condition.<sup>1-3, 26-28</sup> The most common reported symptom is sudden moderate to severe pain, which was initially present in 90 per cent of our patients. Pain was frequently the only symptom (table 2) and in many instances was minimal by the time the patient sought help. Usually substernal, the pain may be associated with pain in the back or epigastrium, occasionally extending to the neck, shoulders, or legs. Substernal or epigastric pain was the

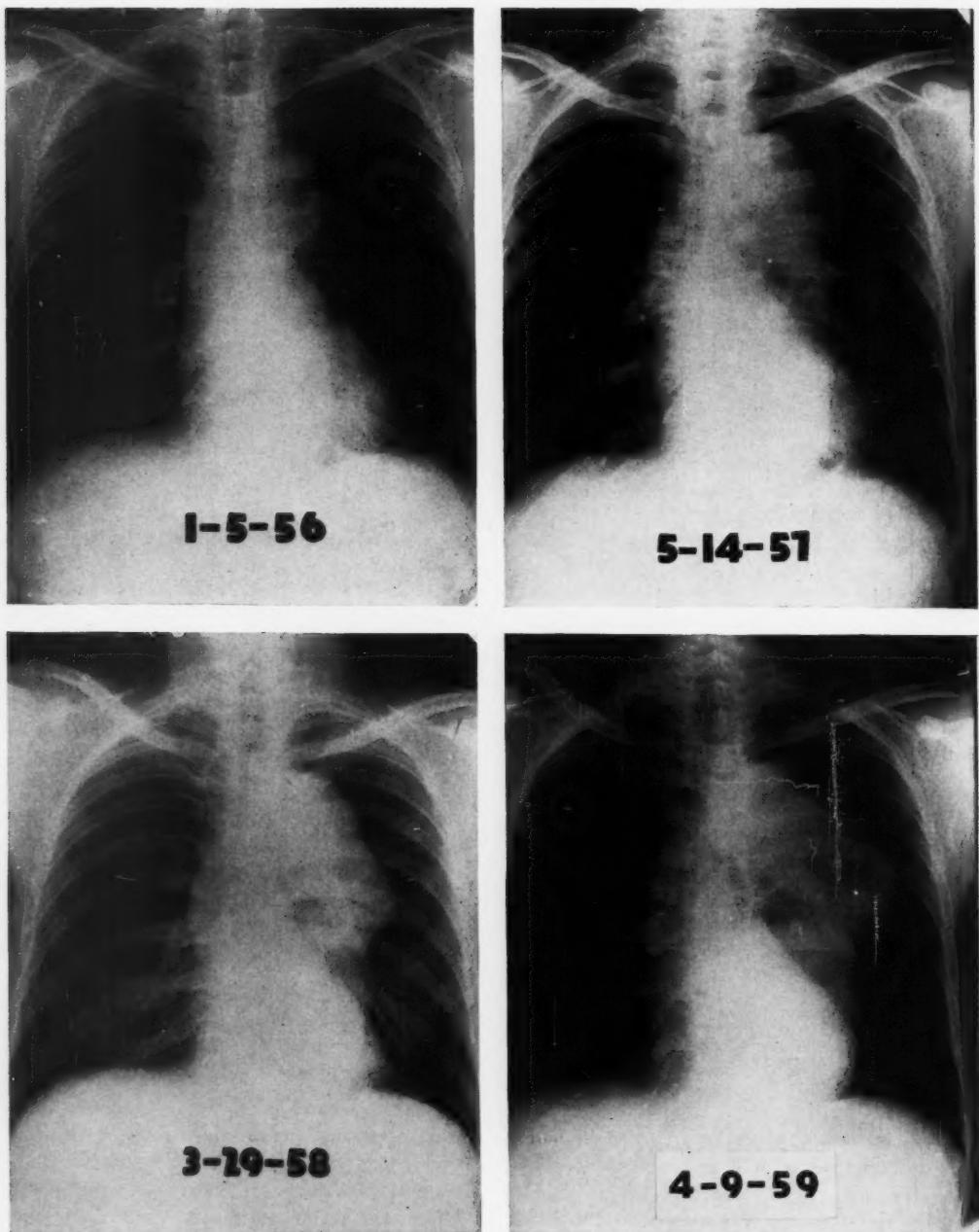


Figure 2A-D

*Serial roentgenograms of the chest demonstrating progressive enlargement during a 3-year period of dissecting aneurysm of the aorta in a 50-year-old physician.*



Figure 2E and F

*Angioaortogram and drawing depicting extent of lesion before operation.*

only symptom in 10 patients. Three of these had been treated for myocardial infarction before the correct diagnosis was obvious. The possibility of a dissecting aneurysm should always be considered in patients being treated for coronary occlusion. During the past 5 years studies of postmortem cases at the Methodist Hospital revealed that three individuals thought to have had myocardial infarction had died suddenly from rupture of a dissecting aneurysm.

Initial shock, reported in about one third of cases,<sup>29</sup> is usually out of proportion to the drop in systolic blood pressure. It may be associated with cyanosis, tachypnea, and tachycardia.

The foregoing are usually the manifestations of acute dissection. Only about one fourth of our patients were treated during this early phase of the dissecting process, i.e., within the first few days to a week after onset of symptoms. The interval between onset of symptoms and operation in the remaining cases ranged from several weeks to 2 years.

The reported incidence of neurologic manifestations ranges from 15 per cent to 46 per cent of cases.<sup>4, 30-33</sup> Three of our patients had suffered from transient paraplegia, two complained of numbness of arm or leg, and two were unresponsive or aphonic for several hours. Neurologic signs or symptoms in patients with thoracic or abdominal pain may be a clue to the early diagnosis of dissecting aneurysm. Certainly neurologic deficits may



Figure 2G

*Photograph at operation of functioning Dacron graft following resection of aneurysm.*

be easily overlooked in the usual examination of a critically ill patient.

Shortness of breath was a complaint of 15 per cent of the patients. Two patients had transient circulatory disturbance of the legs, and hematuria occurred in one instance.

Only two patients were asymptomatic. In one the lesion was discovered by routine roentgenography of the chest and in the other it was discovered incidentally during pneumonectomy.

Two thirds of the patients had associated cardiovascular or other disease. Of these, moderate to severe hypertension of several years' duration was by far the most frequent, being present in 74 per cent of cases. Other complicating conditions included congestive heart failure, arteriosclerotic heart disease, active duodenal ulcer, portal cirrhosis, and gallbladder disease.

The physical manifestations of dissecting aneurysm were not significant in making a

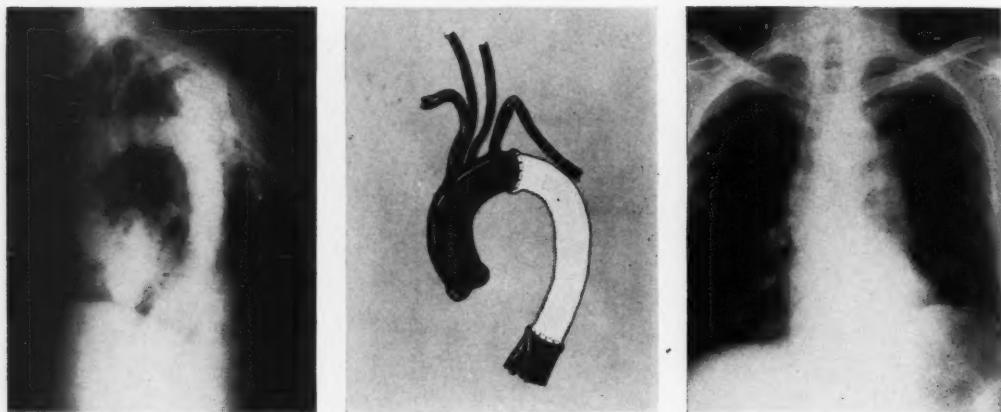


Figure 2H-J

Angioaortogram (left) and drawing (center) depicting satisfactory appearance after resection of descending thoracic aorta, obliteration of distal false lumen, and Dacron graft replacement. Roentgenogram (right) 1 year after operation. The patient has resumed active medical practice.

diagnosis. A precordial apical or basal systolic murmur was present in 40 per cent of cases. A diastolic murmur over the aortic area, which has been emphasized as of diagnostic significance, was present in only eight cases in our series. This probably reflects the small number of patients in our series with involvement of the ascending aorta or aortic

annulus. Brachial blood pressure differential, tracheal deviation, cervical venous distention, abdominal aneurysm, and alteration in peripheral pulses were occasional findings. Fifteen per cent of patients had minimally elevated blood urea nitrogen levels. Myocardial ischemia, left ventricular strain, or rhythm disturbance was demonstrable elec-

Table 3  
*Dissecting Aneurysm of the Aorta. Relationship of Extent of Dissection to Operative Mortality*

AORTIC SEGMENT INVOLVED				Combined Series of Hirst and Shennan		PRESENT SERIES			
Ascending	Arch	Descending	Abdominal	Cases	Per Cent	Cases	Per Cent	Operative Mortality Cases	Per Cent
				342	47	9	12	6	67
TYPE I				212	29	4	6	1	25
TYPE II				115	16	43	60	8	19
TYPE III				54	8	16	22	4	25
TOTAL				723	100	72	100	19	26

Table 4

*Dissecting Aneurysms of Thoracic Aorta. Mortality According to Blood Pressure*

Blood pressure	No. cases	Deaths	
		No. cases	Per cent
149/89 or less	19	0	0
150/90 or more	53	19	36
Total	72	19	26

trocardiographically in 75 per cent of the cases.

Roentgenograms of the chest usually revealed widening of the supraventricular mediastinum and radiolucency of the arch and descending aorta in the region of the false passage. In some instances superior mediastinal widening may be initially insignificant. Progressive enlargement of the aneurysm during a period of several days or months may be observed (figs. 2 A-J). In such instances the risk of death by sudden rupture of the aneurysm is definitely increased. Angioaortogram made with the patient in an oblique position accentuates the "double-barrelled" appearance of the lesion (fig. 3). Angioaortography has been of utmost value in determining the nature and extent of the dissecting process. Mediastinal motion may occasionally produce a similar appearance, as will an organized thrombus lining a long, fusiform arteriosclerotic aneurysm. Calcification of the intima and wall of the false passage producing a double lumen appearance on plain roentgenography of the chest is rarely seen.

#### Pathogenesis and Gross Pathology

The basic lesion of dissecting aneurysm appears to be degeneration of the supporting tissue of the media of the aorta and is apparently unrelated to known pathologic processes, such as arteriosclerosis or aortitis, that commonly involve the aorta.<sup>16, 34, 35</sup> The condition is frequently associated with Marfan's syndrome and hypertension. Medial degeneration with dissection of the aortic wall has been produced experimentally by a diet of *lathyrus odoratus* (sweet pea) meal,<sup>36, 37</sup> prolonged injection of epinephrine,<sup>38</sup> vitamin E deficiency,<sup>39</sup> methionine intoxication,<sup>40, 41</sup> and

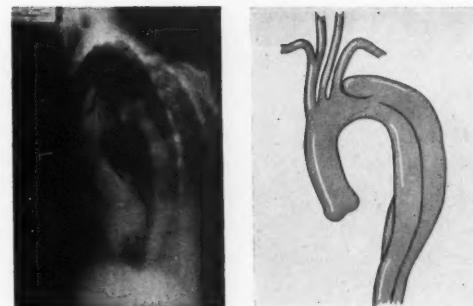


Figure 3

*Angioaortogram with drawing showing salient roentgenographic characteristics of dissecting aneurysm of aorta.*

other means. Many investigators<sup>6, 11, 16, 42-53</sup> believe that the dissecting process is initiated by intimal laceration, whereas others<sup>3, 20, 34, 54-59</sup> are of the opinion that dissection follows rupture of a *vasa vasorum*, the break in the intima occurring secondarily. This is a more plausible explanation for cases of dissecting aneurysm that develop without intimal tear. An intriguing concept of pathogenesis has been offered by Bauer and Hirsch<sup>60</sup> who stated that repeated expansion and contraction of an aortic wall in which there is considerable difference in elasticity of the media and adventitia would result in easy separation of the two layers. Because of unequal elasticity, the shearing force in the wall of the aorta at points of greatest mechanical stress produce dissection without intimal laceration. This incorporates all theories of pathogenesis and explains established observations. It is well known that in the presence of medial degeneration the layers of the aorta can be easily separated.<sup>42</sup> This plane of dissection usually lies between the outer two thirds and inner third of the aortic wall (figs. 4A and 4B). The role of external trauma in the pathogenesis of dissecting aneurysm is uncertain.

Of greater practical importance are the location and extent of the disease. Shennan,<sup>4</sup> Hirst and co-workers<sup>23</sup> divided their cases into four major and 18 minor categories on an anatomic basis. Comparison of our series with theirs indicates the more selective na-



Figure 4A

*Excised portion of thoracic aorta sectioned to show hematoma of media.*

ture of our cases by the greater incidence of the chronic type of lesion (table 3). On the basis of our surgical experience we have classified these lesions into four general categories, since this provides a guide to surgical approach and prognosis (fig. 5).

Type I (12 per cent) includes patients in whom the dissecting process extends distally from the aortic annulus or the aortic arch usually to a point well below the diaphragm (fig. 6). The dissection may involve the carotid, iliac, renal, or mesenteric arteries. Unless there is a localized area where rupture is imminent, resection with graft replacement is of little value. Creation of a re-entry passage is usually preferable and may be done with or without the aid of hypothermia or atriofemoral bypass perfusion.



Figure 4B

*Photograph of dissecting aneurysm of aorta cut in cross section to show plane of dissection located in innermost portion of media.*

In type II (6 per cent) the process is localized to the ascending aorta and proximal transverse arch (figs. 7A and 7B). Operative correction requires utilization of the extracorporeal pump-oxygenator and coronary perfusion to permit excision of the lesion and aortic replacement by graft. In some cases because of dilatation of the aortic annulus and consequent aortic insufficiency, it is necessary to perform an annuloplasty either by wedge resection and suture repair with bicuspидization of the aortic valves or by circumferential suture annuloplasty with graft replacement.

In type III (60 per cent) the dissecting process begins immediately distal to the left subclavian artery and continues well below the diaphragm (figs. 2, 3, and 4). A distal false lumen must be dealt with surgically after the thoracic portion has been excised.

Type IV (22 per cent) is similar to type III except that the dissecting process remains localized to the descending aorta (fig. 8). The entire diseased segment may be excised and replaced by graft.

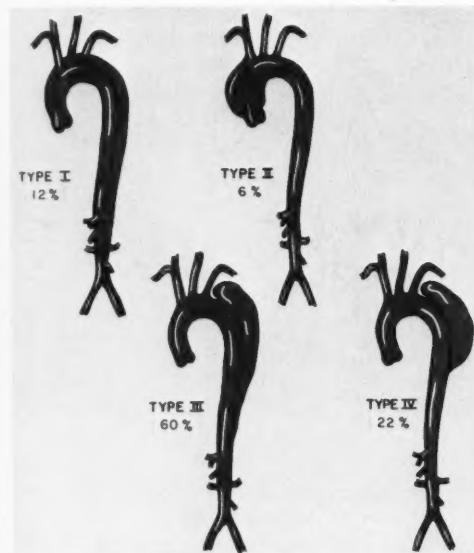


Figure 5

*Surgical classification of dissecting aneurysms of the aorta based on location and extent of lesion.*

Operative correction of the last three types requires use of hypothermia or, preferably, the atriofemoral pump bypass to prevent spinal cord ischemia.

#### Results of Surgical Treatment

Depending on the nature of the lesion three general types of surgical procedure were employed in this series of 72 patients. In only one patient was the dissection so well localized that excision with aneurysmorrhaphy was possible. In five of the earlier patients a re-entry passage was created into the true lumen of the descending thoracic aorta and the distal false lumen was obliterated by suture. The remaining 66 patients had some form of excisional therapy with aortic replacement with the use of left atriofemoral bypass perfusion, external bypass graft, or hypothermia to prevent the cardiac, neurologic, or renal disturbances that follow prolonged occlusion of the descending thoracic aorta. Special technical problems arose in the five patients with involvement of the entire aorta, the one patient who required temporary carotid perfusion, and the six in whom the true lumen was not apparent. The usual procedure was resection

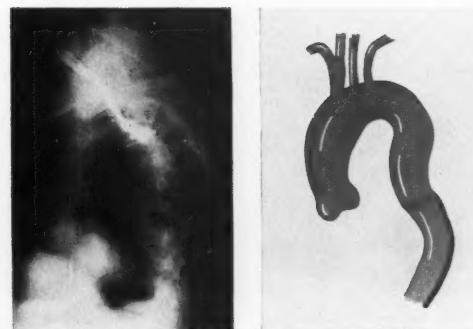


Figure 6

*Angioaortogram and drawing demonstrating appearance after creation of re-entry site in descending thoracic aorta. Patient remained active 4½ years after operation with no evidence of progression of lesion.*

of the descending thoracic aorta so as to include the site of origin of the dissection, obliteration of the false lumen distally, and insertion of a homograft or prosthesis (44 patients) (fig. 9). In 16 patients the entire pathologic process could be excised and replaced by a vascular graft or prosthesis. Of the 59 patients with types III and IV in whom the dissecting process involved the descending aorta, the operative mortality rate was 20 per cent as compared with 26 per cent for the entire group (table 3).

Various materials were used to replace the excised aortic segments. These included aortic homografts in 13 and such synthetic prostheses as Ivalon in four, Nylon-Dacron in six, Daeron in 24, Dacron-Ivalon in 1, and Teflon in 18 cases. Although each prosthesis has its own special characteristics, closely woven fabrics have proved most satisfactory for aortic replacement in patients requiring heparinization. Only one death was attributable to failure of the graft; this death occurred 2 years after operation from rupture of a dissecting aneurysm of the homograft. Homografts have functioned satisfactorily in the thoracic aorta for more than 7 years after insertion. Our first synthetic prosthesis in this series has functioned satisfactorily for more than 4 years.

Under hypothermic conditions the diseased segment in 11 patients was resected and the

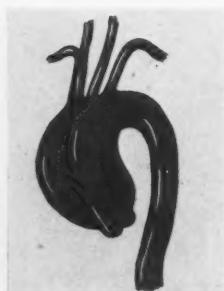


Figure 7A

*Angioaortogram and drawing revealing unusual dissecting aneurysm localized to ascending aorta.*

defect replaced by a graft. The duration of aortic occlusion ranged from 19 to 87 minutes. Permanent paraplegia developed in only one patient, cooled to 90 F. with interruption of aortic flow for 63 minutes.

With use of left atriofemoral bypass perfusion, 42 lesions were resected with aortic replacement (fig. 9). The duration of occlusion ranged from 28 to 102 minutes, averaging 49 minutes. Two patients were paraplegic immediately after operation; however, one whose occlusion time was 42 minutes completely recovered after 3 weeks. The other, whose occlusion time was 102 minutes, had permanent neurologic changes.

In two instances localized difficulties caused grafts to be inserted without benefit of bypass or hypothermia. Transitory paresthesias developed in the legs of one of these after 34 minutes of aortic occlusion, whereas the other after 37 minutes of occlusion, had no complication.

In five re-entry procedures no sequelae occurred that could be attributed to temporary occlusion of the descending aorta during the anastomosis, which averaged less than 30 minutes. Interruption of blood flow in the aorta at the midportion of the descending thoracic aorta for such a short period carries a low risk of neurologic damage.

Three patients had a permanent bypass and obliterative endoaneurysmorrhaphy. One patient with a large tortuous dissecting aneurysm extending from the left subclavian artery to the midportion of the abdominal aorta had a permanent external Dacron by-

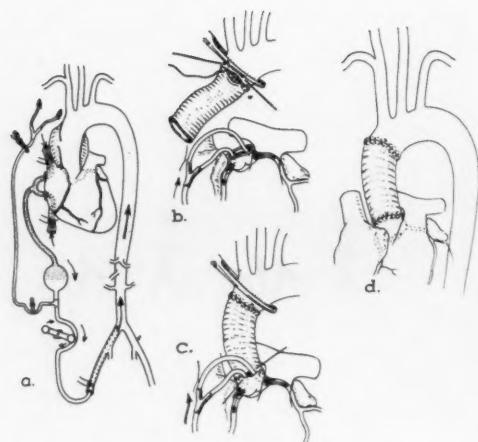


Figure 7B

*Drawings illustrating technic or surgical excision of this type of aneurysm with Dacron graft replacement utilizing extracorporeal pump oxygenator and coronary perfusion.*

pass from the distal aortic arch to the lower abdominal aorta (fig. 10). After the bypass was functioning satisfactorily, the entire descending thoracic aorta was excised and the two lumens were oversewn at the diaphragm. This patient did well until massive upper gastrointestinal hemorrhage from acute gastric ulcerations required emergency gastrectomy on the eighth postoperative day. He died several days later from myocardial infarction. A second patient died from renal failure following a transfusion reaction 9 days after operation, and a third patient is living and well after obliteration of the aneurysm, which extended from the left subclavian to the celiac artery.

A brief analysis of the 19 operative deaths reveals an average postoperative survival time of 10 days. Six patients died of rupture of the aneurysm, three rupturing into the pericardial sac producing tamponade. Four patients died in congestive heart failure, and three died of renal insufficiency. In three instances cardiac arrest occurred at operation or immediately thereafter. The remaining three patients died of myocardial infarction, pulmonary embolism, and cerebral infarction, respectively.

Three of the 11 patients who had resection

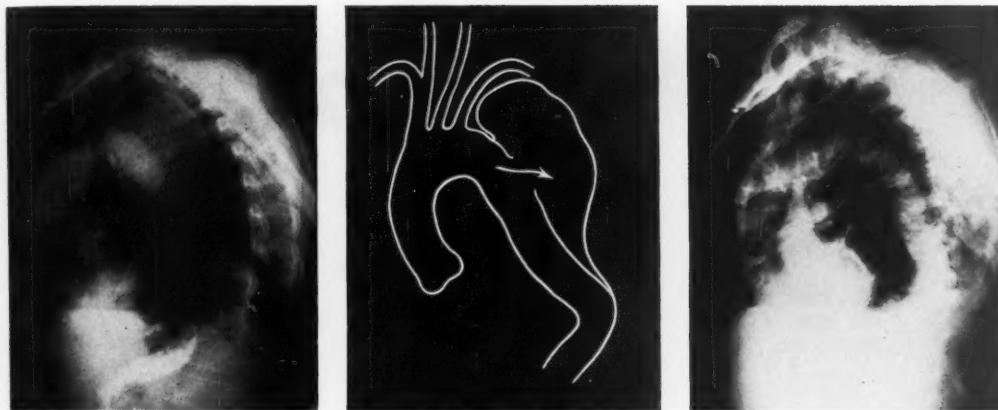


Figure 8

Left. *Angioaortogram*. Center. *Drawing depicting large dissecting aneurysm limited to descending thoracic aorta*. Right. *Angioaortogram after operation*.

under hypothermic conditions died (27 per cent). Eleven deaths occurred in the bypass perfusion group (23 per cent), and three of four patients who had creation of a re-entry passage died. Advanced age per se did not alter the prognosis. Hypertension appears to be the most significant factor in relation to operative risk as there were no deaths in the normotensive group (table 4).

With few exceptions the results in the 53 patients surviving operation, in all of whom follow-up studies have been made, have been gratifying. Follow-up observations for more than 5 years reveal that most of these patients have resumed normal activities. Many are retired but active, but others engage in hard manual labor. One patient is a semi-invalid, and two are paraplegic. Five patients have subsequently had aneurysms of the abdominal aorta resected successfully with graft replacement (fig. 11).

Only six patients have died since discharge from the hospital. In one fulminating hepatitis developed, presumably from blood transfusions, and the patient died 1 month after operation. Two succumbed to intracranial hemorrhage or hypertensive cardiovascular disease 10 months and 12 months after operation, respectively. One man committed suicide 1 year later. Two patients died of internal hemorrhage after 2 years, one from rupture

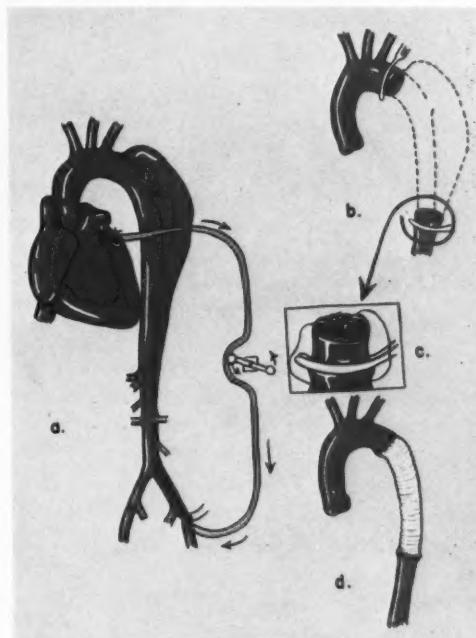


Figure 9

*Drawings illustrating technic of left atriofemoral bypass perfusion permitting excision of descending thoracic aneurysm with minimal risk of neurologic sequelae.*

of an aneurysm of the innominate artery and the other from a rupture of a dissecting aneurysm of the homograft.

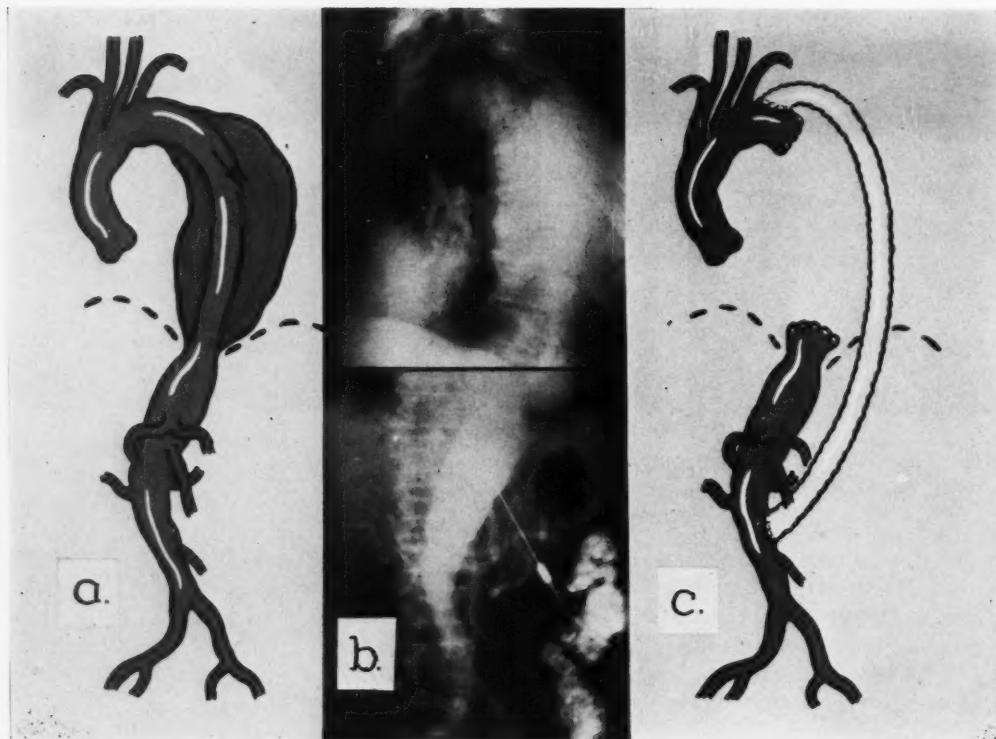


Figure 10

A.-C. Drawings and photographs showing technic of excision of large dissecting aneurysm of descending thoracic and abdominal aorta utilizing permanent Dacron bypass graft extending from aortic arch to distal abdominal aorta.

Follow-up roentgenograms of the chest and angiograms have failed to demonstrate progression of the disease or unsatisfactory appearance of the homografts or prostheses for periods up to 5 years. One physician, alive more than 5 years after creation of a re-entry passage, has had no further progression of the lesion (fig. 6).

#### Comment

Until recently, treatment of dissecting aneurysms of the aorta was largely symptomatic and had little or no effect upon the highly fatal course of the disease. This is well illustrated by the reports of a number of observers. In the classic study of Shennan,<sup>4</sup> for example, 65 per cent of the patients died within 24 hours after onset, and an additional 26 per cent died within 1 day to 1 week. More re-

cently, Hirst and associates<sup>23</sup> in a study of the survival period of 425 patients found that 74 per cent died within 2 weeks after onset and 89 per cent within 3 months. Other investigators have had somewhat similar experience. This indicates that in general only about 10 to 15 per cent of patients have the chronic "healed" type of dissection. Even in this group, however, further progression of the dissecting aneurysm frequently takes place and ultimately leads to fatal termination.

In light of these observations emphasizing the extremely grave nature of this disease, more aggressive therapy directed toward altering the natural highly fatal course of the disease is definitely indicated. That this can be done by application of surgical treatment is evidenced by comparing the significantly greater survival rate in our operative series

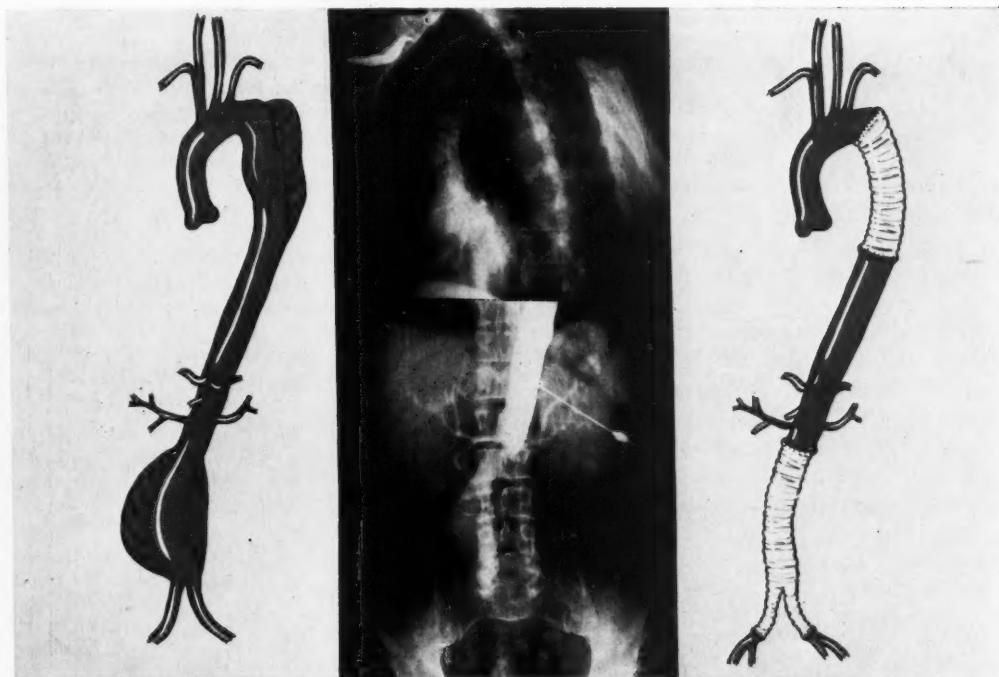


Figure 11

Drawing (left) and angioaortogram (center) with abdominal aortogram before resection of dissecting aneurysm of descending thoracic aorta and before resection of a fusiform aneurysm of abdominal aorta. Diagrammatic appearance of aorta (right) after resection of thoracic and abdominal aneurysms.

with that of Hirst and associates in their non-operative series (fig. 12).

Many variations exist in the pathologic features of the disease, particularly in regard to location and extent of the dissecting process. Since the surgical approach to these various types of lesions may require certain modifications, it is essential to determine preoperatively as precisely as possible the origin, location, and extent of the dissecting process. In most instances this can be done by special angiographic studies. Our experience would suggest that the most favorable lesions for operative treatment are those in which the dissecting process originates in the descending thoracic aorta distal to the left subclavian artery. Fortunately these are the types of lesions that tend to occur in patients who survive the initial episode for more than a few days and thus permit sufficient time for application of surgical treatment.

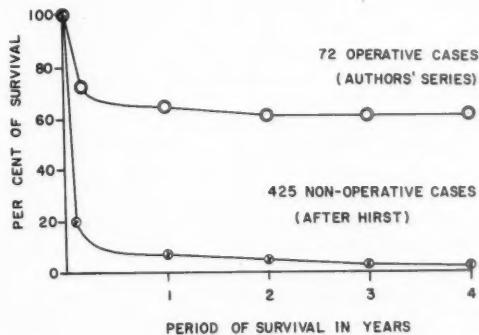


Figure 12

Survival rate of operative and nonoperative cases of chronic dissecting aneurysm of aorta.

#### Summary

Dissecting aneurysm of the aorta is an extremely serious condition that pursues a rapidly fatal course in more than 75 per cent of cases. Follow-up data on 72 patients surgi-

eally treated indicate that effective surgical treatment significantly alters the course of the disease and in the majority of instances removes the threat of death by rupture. The operative mortality rate in these 72 patients was 26 per cent. For lesions occurring distal to the left subclavian artery, resection of the descending thoracic aorta with replacement by aortic graft utilizing hypothermia or the bypass pump was successful in 80 per cent of cases.

Most patients surviving operation have been able to resume previous activities with minimal risk of sudden death from rupture of the aneurysm or failure of the aortic graft.

The importance of recognizing the characteristic clinical manifestations, of making precise roentgenographic diagnosis, and of instituting effective surgical treatment in the management of dissecting aortic aneurysm has been stressed.

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## ABSTRACTS

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### ELECTROCARDIOGRAPHY, VECTORCARDIOGRAPHY, BALLISTOCARDIOGRAPHY, AND OTHER GRAPHIC TECHNICS

Douglas, A. H., and Samuel, P.: Analysis of Electrocardiographic Patterns in Hypothyroid Heart Disease. *New York State J. Med.* 60: 2227 (July 15), 1960.

An analysis of the electrocardiograms of 92 patients with hypothyroidism was made. Only those individuals in whom the clinical diagnosis was confirmed by basal metabolism, protein-bound iodine, blood cholesterol levels, or radioactive iodine uptake, and in whom there was both clinical and electrocardiographic response to thyroid extract therapy were used. Seventy of the 92 patients were women. Electrocardiograms obtained after correction of the hypothyroidism were compared with those taken before treatment. The average heart rate was 68 beats per minute before treatment and 100 beats per minute after treatment. The axis of the P wave in the frontal plane was not modified by hypothyroid heart disease. The average voltage of the P wave was higher in every lead after treatment, but the increase in voltage was statistically significant only in leads I, II, III, aV<sub>R</sub>, aV<sub>L</sub>, and V<sub>4</sub>. The P-R interval was not modified by hypothyroid heart disease. The axis of the QRS complex in the frontal plane was not influenced substantially by the disease. Although the average voltage of the R wave increased in all leads after control of hypothyroidism, the difference was significant in leads I and II only. The sum

of the R and S waves was significantly increased in leads I, II, III, V<sub>3</sub>, and V<sub>4</sub>. The zone of transition was seen more often on the left side (between V<sub>4</sub> and V<sub>5</sub>) before treatment than after treatment. The S-T segment remained unchanged in acquired hypothyroid heart disease. The most important changes involved the T wave. In 44 per cent of the patients the frontal axis of the T wave was not situated in the first quadrant. This change was reversible by specific therapy. In the limb leads the increase in voltage of the T wave was significant in leads I, II, and III. In the right-sided precordial leads (V<sub>1</sub> to V<sub>4</sub>) the T wave was inverted, flat, or diphasic in the majority of patients before treatment. After treatment there was a significant change toward normal in these positions and the average T wave became upright in leads V<sub>1</sub> to V<sub>4</sub>. This is in contrast to the T-wave pattern in leads V<sub>5</sub> and V<sub>6</sub>. In the left-sided precordial positions the average T wave was positive before treatment and there were no significant changes after treatment. When T-wave inversion appears in leads V<sub>1</sub> to V<sub>4</sub> the possibility of hypothyroid heart disease should be considered. This pattern is more likely to be reversed by the administration of thyroid extract than is the pattern of T-wave inversion in leads I, aV<sub>L</sub>, V<sub>5</sub>, and V<sub>6</sub>. Because of its relative irreversibility, the latter pattern suggests complicating coronary artery disease when it is found in patients with hypothyroidism, and thyroid extract should be used more cautiously in this group.

KRAUSE

**Hiss, R. G., Lamb, L. E., and Allen, M. F.: Electrocardiographic Findings in 67,375 Asymptomatic Subjects. X. Normal Values.** Am. J. Cardiol. 6: 200 (July), 1960.

Normal electrocardiographic values were computed from the 12-lead directly inscribed electrocardiograms of 6,014 healthy Air Force flying personnel aged 16 through 58 years. P-wave duration ranged from 0.06 to 0.15 second, amplitude from 0.05 to 0.35 mv. and axis from -30 through +90 degrees with 95 per cent of the P axes falling between +15 and +75 degrees. P-R interval was arbitrarily limited to 0.20 second and ranged down to 0.10 second. QRS duration ranged from 0.05 to 0.11 second, QRS axis from -90 to +120 degrees. QRS amplitude could not be related to body weight. The R/S ratio was greater than 1 in lead V<sub>1</sub> in 0.7 per cent of subjects and in lead V<sub>2</sub> in 8.5 per cent. The mean spatial QRS-T angle ranged between 0 and +70 degrees in 96 per cent of the group. The Q-T interval varied from 0.24 through 0.47 second with a median of 0.38. T-wave amplitude was listed statistically, and in an adjoining paper the interpretation of inverted T waves in healthy individuals was discussed. S-T elevation was noted in 91 per cent of the series and ranged between 0.1 and 0.5 mv. in amplitude. Very few Q waves of 0.04 second duration were found and all of these were in lead III. Older individuals seemed to have slower heart rates, longer Q-U intervals, less pronounced S-T elevation, and lower T waves.

ROGERS

**Jackson, B. T., Clarke, J. P., and Egdahl, R. H.: Direct Lead Fetal Electrocardiography with Undisturbed Fetal-Maternal Relationships.** Surg., Gynee. & Obst. 110: 687 (June), 1960.

A technic for the individual recording of fetal and maternal electrocardiograms in the rabbit is described. At 3 to 4 weeks gestation the uterus was exteriorized through an abdominal incision and three tiny arrowhead electrodes were embedded in the respective limbs of the fetus. These were connected to a two-channel Grass electroencephalograph for the recording of the three standard limb leads at a standardization of 2.15 em. per millivolt. The resulting tracings clearly showed P, QRS, and T waves, and S-T segment deviation without maternal electrocardiographic superimposition. They were repeatedly obtained following replacement of the uterus into the abdomen and for as long as 24 hours thereafter.

ROGERS

**Karni, H. S.: The Diagnostic Significance of the Differential Repolarization of the Precordial Leads.** Cardiologia 36: 257, 1960.

Phase shift of repolarization was studied in 96 normal subjects and in eight patients with right ventricular hypertrophy. Various precordial lead positions, both conventional and unconventional, were chosen as exploring electrodes with V<sub>1</sub> as a reference lead. In 25 per cent of the normal subjects, T waves recorded with these exploring electrodes showed a phase shift from those of V<sub>1</sub>, which changed in degree with the slightest movement of the exploring electrode. This phase shift was found in normal subjects only over a limited area coinciding with that of absolute cardiac dullness. On the other hand, in the patient with right ventricular hypertrophy, this phase shift could be detected all over the right ventricle, and it was less affected by small motion of the exploring electrode. It was concluded that this method permitted diagnosis of myocardial injury and early lesions of ventricular hypertrophy.

BRACHFELD

**Lancaster, W. M., Semple, T., and Kelly, J. C.: ABC Leads in Ischaemic Heart Disease.** Brit. Heart J. 22: 347 (June), 1960.

The authors evaluated the ABC leads of Trethewie using a simplified method of lead placement in the frontal, horizontal, and sagittal planes. Left ventricular hypertrophy and strain were well demonstrated in lead B; arrhythmias and bundle-branch block were satisfactorily represented. Certain criticism became apparent; thus respiratory variations and somatic tremor were common and the R wave in all leads tended to be small in amplitude, so that the significance of an absent R wave was difficult to evaluate. The authors were unable to confirm about one third of the posterior infarctions and ischemic changes by use of lead A when such changes were clearly demonstrated by the conventional 12-lead electrocardiogram. Only two patients were found who demonstrated septal ischemic abnormalities in the ABC leads with normal conventional electrocardiograms. The authors doubt the value of this method in diagnosis.

KALMANSOHN

**Lewis, B. M., Sokoloff, L., Wechsler, R. L., Wentz, W. B., and Kety, S. S.: A Method for the Continuous Measurement of Cerebral Blood Flow in Man by Means of Radioactive Krypton (Kr<sup>79</sup>).** J. Clin. Invest. 39: 707 (May), 1960.

A method is described whereby the uptake of Krypton<sup>79</sup> by the brain is followed by measuring directly the gamma radiation through the intact

skull. With this technic it is possible to measure continuously the cerebral blood flow during transient or rapid changes before equilibration between brain tissue and cerebral venous blood is achieved. Mean total cerebral blood flow in 10 normal young men was 1,236 ml. per minute, with a standard deviation of 246 ml. per minute. The cerebral blood flow determined by this method was found to be significantly higher than the values obtained simultaneously by the nitrous oxide method, but the results of the two methods were, however, significantly correlated. The discrepancy was thought to be due to the collective effects of a number of factors such as the extra radiation picked up from the extracerebral tissues or the disproportionate weighting of different areas of the brain in the total head count. The Krypton<sup>70</sup> method was found to be capable of following rapid changes of cerebral blood flow such as occur during hyperventilation, carbon dioxide inhalation, or norepinephrine infusion.

KARPMAN

**Manning, G. W.: An Electrocardiographic Study of 17,000 Fit, Young Royal Canadian Air Force Aircrew Applicants.** Am. J. Cardiol. 6: 70 (July), 1960.

Electrocardiograms were routinely obtained over the past 11 years from 17,000 aircrew applicants aged 18 to 24 years. Nine hundred and fifty-four showed abnormalities, causing the individual to be rechecked by a cardiologist. Of these, 86 applicants were rejected for pilot training on the basis of the disorder discovered electrocardiographically, and in half of this group no other evidence of cardiovascular disease was elicited. The disqualifying abnormalities comprised T-wave changes in 25, anomalous atrioventricular excitation in 22, bundle-branch block in 19, P-R interval greater than 0.24 second in 6, and other aberrations in 14. A follow-up study of airmen studied electrocardiographically at the beginning of World War II was in progress, and it was thought that a more objective basis for electrocardiographic qualification for flying may be forthcoming. The routine use of electrocardiography was advocated in the selection of pilot trainees.

ROGERS

**Moia, B., Otero, E. A., Muchnik, J., Alvarez, A. J., and Cecchi, A.: Effect of Provoked Elevation of Right Atrial Pressure on the Value and Configuration of Right Ventricular Pressure Curves.** Arch. mal. coeur 53: 186 (Feb.), 1960.

In 25 patients abdominal compression was carried out during registration of right atrial and ventricular pressures by cardiac catheterization. Patients with past or present right heart failure showed an elevation of more than 2 mm. Hg in the right atrial pressure, and a marked elevation of right ventricular diastolic pressure, which could exceed one third of systolic pressure. At the same time, the configuration of the pressure curve approached that characteristic of constrictive pericarditis. The radiologic heart shadow usually showed an increase in size at the expense predominantly of the left cardiac cavities. In patients without right heart failure abdominal compression caused no significant changes. The abdominal compression test was considered the most valuable test in the diagnosis of compensated or noncompensated right ventricular failure in patients whose right intracardiac pressures and pressure curves were normal or only slightly abnormal. This test can also be of use for the purpose of increasing the atrioventricular pressure gradient in the diagnosis of tricuspid stenosis.

LEPESCHKIN

**Morrow, A. G., Braunwald, E., and Ross, J., Jr.: Left Heart Catheterization.** AMA Arch. Int. Med. 105: 645 (Apr.), 1960.

Seven basic technics for left heart catheterization are described along with their advantages and disadvantages. In transbronchial left heart catheterization access to the left atrium is gained by puncture of the anterior bronchial wall by a special needle passed through a bronchoscope. No fatalities or serious complications were observed. It is not suited to children, since general anesthesia is required and problems of ventilation and relaxation are encountered. In transseptal left heart catheterization a catheter is introduced into the saphenous vein and on into the right atrium, a special needle is then pushed through the catheter, punctures the septum, and enters the left atrium. This procedure has the advantage of allowing both left and right heart catheterizations to be carried out with the patient under basal conditions. It is very suitable in children and when prolonged observation of left heart pressures are desired. In posterior percutaneous left atrial puncture a needle is inserted into the right posterior chest wall at a level corresponding to the position of the left atrium determined fluoroscopically. The needle is then directed obliquely toward the midline and penetrates the left atrium. Although technically simple, the method is becoming unpopular because of numerous severe complications that have occurred; these include intrapericardial

bleeding, cardiac tamponade, pneumothorax, and most frequently hypotension. In anterior left ventricular puncture the left atrium is directly punctured through the anterior chest wall. It is simple, safe, and useful in evaluating patients with acquired or congenital aortic stenosis, especially infants and children. Also, it permits the injections of dyes or radioisotopes for detection and localization of circulatory shunts. In retrograde left ventricular catheterization the left ventricle is entered via a catheter passed in a retrograde fashion through the femoral or right brachial artery, either percutaneously or through an arteriotomy. This technic is of value in localizing the site of left ventricular outflow. The usefulness of suprasternal left atrial puncture is limited to studies in which only pressure in the left atrium and great vessels is desired. Catheterization of the left heart through intracardiac communications remains a useful and safe technic in patients with congenital heart disease and a defect in the interatrial or interventricular septum. Injection of dyes or radioisotopes are important in characterizing congenital anomalies, and selective angiocardiography by this route is of great value in preoperative evaluation of patients with various types of left-to-right shunts. All of these methods have advantages, and frequent combinations of methods are used depending upon the type of patient, experience of the investigator, and facilities available.

KRAUSE

**Nilsson, P. E.: An Examination of the Electrocardiogram as Recorded by Slapak and Partilla's Chest Leads in Normal Persons and in Patients Suffering from Myocardial Infarction.** Acta med. scandinav. 166: 487, 1960.

The Slapak and Partilla chest leads are recorded in bipolar fashion by placing an electrode at the left posterior axillary line at the level of the apex and the other electrode at four different positions on a line from the left second intercostal space at the sternal margin to the left anterior axillary line. These leads are said to be superior to the usual electrocardiographic techniques in the diagnosis of posterior myocardial infarction. The material included 30 normal subjects, 30 patients with myocardial infarction, of whom four died and were autopsied, and a further five autopsies of patients who had never had a coronary incident. There was so great a variation among normal persons that it was difficult to form normal limits to compare with those of the original authors. In patients with posterior infarction, the changes were similar to those described by Slapak and Partilla; but in a few, characteristic changes were missing in the

special leads even though they were present in the usual ones. The findings in subjects with anterior infarction were similar. Some of the patients who were autopsied and who did not have myocardial infarction, either clinically or at post mortem, showed typical changes in the Slapak and Partilla leads. Because of this variability, it can only be concluded that the Slapak and Partilla leads are less reliable than the standard electrocardiograms.

SHEPS

**Sangiorgi, M., Corsi, V., Cofano, L., Salvo, E., and Coppolino, L.: A Comparative Study of Some Vectorcardiographic Methods Based on the Use of Unipolar Leads, Bipolar Leads between Symmetrical Points and Bipolar Leads with Multiple Electrodes.** Acta cardiol. 15: 101, 1960.

Results of a comparative study of frontal, horizontal, and sagittal vectorcardiograms recorded with three different derivation systems are described: a unipolar derivation system placed in the orthogonal planes passing through the point O, the electric center of the heart (Jouye); a bipolar derivation system arranged in analogue planes between opposite points of the electric symmetry and a bipolar derivation system with multiple electrodes (Helm). In a few instances the system of McFee and Johnston as well as the cube system (Grishman) were also studied. In many normal and pathologic cases, after having adjusted the orthogonal components (according to the method of Jouye), the unipolar system was similar to the bipolar system with derivations between symmetric points. In addition, there was, in about 80 per cent of normal and pathologic cases studied with both these systems, a good correlation with Helm's multiple electrodes system. A convincing relationship was observed between all three of these systems and between that of McFee and Johnston, but not with that of Grishman. The advantages offered by each of the three systems are discussed from the point of view of the corrected vectorial representation of the electric activity of the heart.

BRACHFELD

**Schelling, J. L., Reymond, Cl., and Rivier, J. L.: Simultaneous Recording of Phonocardiogram and Apex Cardiogram.** Cardiologia 36: 199, 1960.

By simultaneous records of phonocardiogram and apex beat it is possible to recognize in the former the opening of the atrioventricular valves and the phase of rapid ventricular filling in early diastole. In mitral stenosis the apical cardiogram

shows certain abnormalities. Some examples from cases of mitral and tricuspid valvular disease are given in order to illustrate the method which supplements arterial and venous pulse tracings.

BRACHFELD

**Ungerleider, H. E.: The Prognostic Implications of the Electrocardiogram.** Am. J. Cardiol. 6: 35 (July), 1960.

The value of the electrocardiogram in prognosticating mortality among life insurance employees or clients was discussed. Mortality rates were calculated or estimated to be increased three- to five-fold (ratio of 3 to 500 per cent) or more following recovery from myocardial infarction. Severe angina pectoris had a mortality ratio of 500 per cent when associated with a major electrocardiographic abnormality, but the rate was 2 to 300 per cent when the double two-step exercise test was negative. Pronounced T-wave abnormalities had ratios of 2 to 500 per cent, while minor T-wave lowering was less ominous. Complete block of either the right or left bundle-branch gave a ratio of 200 per cent when unaccompanied by other obvious evidence of cardiovascular disease. Premature beats were associated with organic heart disease in over half of 1,142 insurance applicants, and, in another study, a high mortality ratio was found. Hypertensive subjects with normal electrocardiograms had a ratio of 186 per cent and those with abnormal tracings had 344 per cent. The electrocardiogram therefore was regarded as having considerable prognostic value in terms of duration of life.

ROGERS

**Widimsky, J., Valach, A., Dejdar, R., Fejfar, Z., Vyslouzil, Z., and Lukes, M.: The Electrocardiographic Pattern of Right Ventricular Hypertrophy in Cor Pulmonale (due to Pulmonary Tuberculosis.)** Cardiologia 36: 287, 1960.

The electrocardiogram was correlated with the pulmonary arterial pressure at rest and during exercise in 80 patients with chronic pulmonary tuberculosis. Of this group 23 had pulmonary hypertension at rest (mean pulmonary arterial pressure greater than 20 mm. Hg). Among the 57 patients with a pulmonary arterial pressure (PAP) smaller than 20 mm. Hg at rest, the PAP during exercise was assessed in 36 individuals; 23 had pulmonary hypertension during light exercise and 13 had normal PAP during exercise. A positive electrocardiographic diagnosis of right ventricular hypertrophy was made in only 30.4 per cent of patients with pulmonary hypertension at rest and in 17.4 per cent of patients with

hypertension during exercise. A probable diagnosis ("indirect" signs) was made in an additional 39.2 per cent of patients in whom pulmonary hypertension occurred during exercise.

BRACHFELD

### ENDOCARDITIS, MYOCARDITIS, AND PERICARDITIS

**Lhotka, J.: Results of Surgical Treatment of Constrictive Pericarditis** Cor et Vasa 2: 38, 1960.

Of 33 patients with constrictive pericarditis subjected to pericardectomy, six patients with concomitant mitral disease or myocardial infiltration died of intractable postoperative ventricular dilatation. In these patients too large an area of pericardium was removed. The results were excellent in 27 patients, persisting up to 9 years. In all patients the left anterior transpleural route was used. In most instances pericardectomy was restricted to the ventricles, and in some patients to the area of the apex only.

LEPESCHKIN

**Oka, M., Nakao, K., and Angrist, A.: Nonspecific Aspects of Endocarditis.** New York State J. Med. 60: 669 (Mar. 1), 1960.

Even though group A streptococci are involved in the pathogenesis of rheumatic endocarditis, nonspecific factors are also concerned in the production of such valvular lesions. The experimental production of the basic lesions of valvular vegetations by nonspecific stress mechanisms is demonstrated. This may occur through an alteration of the endocrine system, particularly the pituitary-adrenal axis. It is suggested that group A streptococcal infection may seed an initial nonbacterial infection and, hence, therapy and prophylaxis are correctly directed to the control of streptococcal infection. However, perhaps all forms of nonspecific stress should also be avoided. Ultimately reestablishment of the normal endocrine balance to control reaction of the connective tissue of the valve may prove to be a fundamental approach in the avoidance of progressive valvular distortion.

KRAUSE

### HYPERTENSION

**Beraldo, W. T.: Rat Blood Pressure as a Diagnostic Procedure for Pheochromocytoma.** Arch. Int. Pharmacodyn. 126: 37 (June), 1960.

A method of assay for urinary catecholamines by using the rat's blood pressure was described. The pressor effect of urine samples from four patients suffering from pheochromocytoma was

compared against a standard of norepinephrine and the activity ranged from 0.66 to 1  $\mu$ g per ml. The sensitivity of the test was such that the output of pressor amines in the urine of patients with pheochromocytoma could be estimated but not that of normal persons.

BRACHFELD

**Elliott, P. M.: Intravenous Protoveratrine in the Prevention and Management of Eclampsia.** *J. Obst. & Gynaec. Brit. Emp.* 66: 610 (Aug.), 1959.

The author found intravenous protoveratrine an effective agent in the management of pregnancy complicated by a marked rise in blood pressure. The route of administration depended on the degree of hypertension. Oral therapy was indicated for mild, intramuscular for moderate, and intravenous for severe hypertension. After initial rapid reduction of blood pressure to a desired level by intravenous protoveratrine, maintenance with intramuscular or oral preparations was continued. A series of 25 cases is described. Four patients had essential hypertension with superimposed preeclampsia, and there was one patient with chronic renal disease. "Puroverine" (Sandoz P. V. S. 295) was administered to 17 antepartum and eight postpartum cases. One ampoule (0.1 mg./ml.) was dissolved in 10 ml. of distilled water or 5 per cent glucose and injected at the rate of slightly more than 1 ml. per minute until a fall of about 30 mm. Hg systolic and 25 diastolic occurred. The blood pressure usually continued to fall, but a second injection may sometimes be necessary. The main pharmacologic actions of protoveratrine are a hypotensive response, bradycardia, and a digitalis-like effect on the failing heart. Control of vasoconstriction improves cerebral, renal, and uterine circulation, alleviates signs and symptoms, and prevents serious sequelae. Response was more rapid in some patients, but control of convulsions was prompt. Headache was relieved in 4, scotoma in 1, and vomiting in 1.

MAXWELL

**Laragh, J. H., Ulick, S., Januszewicz, V., Deming, Q. B., Kelly, W. G., and Lieberman, S.: Aldosterone Secretion and Primary Malignant Hypertension.** *J. Clin. Invest.* 39: 1091 (July), 1960.

A trace dose of tritiated aldosterone was injected into hypertensive and normotensive (control) subjects and the specific activity of labeled tetrahydroaldosterone was determined in the subsequent 24-hour urine specimen. By this method the adrenal secretion rate of aldosterone was

found to be within normal limits in eight normal subjects, in eight patients with benign essential hypertension, and in two patients with hypertension due to unilateral renal disease. Significantly elevated adrenal secretion rates of aldosterone were found in five patients with proved aldosteronism, in three of eight patients with advanced hypertension, and in 14 out of 15 patients with malignant hypertension. The authors conclude that aldosterone hypersecretion does not appear to participate in the pathogenesis of primary (benign) hypertensive disease but that it may be a causal factor in the development of the malignant syndrome. In addition, they hypothesize that the hypersecretion of aldosterone may be due to bilateral renal hyperfunction in patients with malignant hypertension, whereas only a single adrenal adenoma is usually present in cases of primary aldosteronism.

KARPMAN

**Lefebvre, R., and Genest, J.: Study of Renal Ischaemic Tubular Atrophy in 79 Patients with Arterial Hypertension.** *Canad. M. A. J.* 82: 249 (June 18), 1960.

Histologic studies of the kidneys were carried out in 71 autopsied hypertensive patients and in eight patients having had a nephrectomy because of hypertension. In the former group, the ages ranged from 19 to 75 years, averaging 50, and 56 per cent were women. The hypertension was essential in type in 57 patients, of renal origin in 14; neither its duration nor its severity was stated. Atrophy of the cortical tubules was found in 63 per cent of cases. It was frequently spotty and, even when severe, was occasionally unilateral. More severe atrophy was observed in younger patients; it was associated with greater reduction in total kidney weight, in kidney function test results, and with severe retinopathy. The atrophy was more pronounced and was consistently present in hypertension of renal origin, but its severity did not correlate well with the presence of pyelonephritis. It was related to ischemia and correlated closely with the degree of renal arteriosclerosis. The significance of ischemic tubular atrophy in hypertensive patients was still thought to be uncertain.

ROGERS

**McQueen, E. G., and Morrison, R. B. I.: The Hypotensive Action of Diuretic Agents.** *Lancet* 1: 1209 (June 4), 1960.

The relation between the diuretic and hypotensive effects of chlorothiazide and hydrochlorothiazide was investigated in nonedematous hyper-

tensive patients, and the effects of the two drugs were compared with those of a mercurial diuretic. After treatment with any of these drugs for 3 days, there was a reduction in blood pressure. There was no significant difference between the blood pressure fall produced by chlorothiazide or hydrochlorothiazide and that produced by mersalyl. Analysis of regression did not demonstrate, in the group as a whole, a significant relation between the blood pressure fall and the increase in fluid, sodium, or potassium excretion, or between blood pressure fall and initial blood pressure. In 13 patients in whom the effects of hydrochlorothiazide and mersalyl were compared at the same blood pressure level, a characteristic diuretic response to either agent was accompanied by a characteristic blood pressure response. The blood pressure fall was not correlated with plasma-volume contraction, but was probably significantly related to a reduction in the thiocyanate space. It is suggested that chlorothiazide reduces blood pressure by its diuretic action and that this drop is characteristic of the individual rather than the drug. This fall induced by diuresis is brought about in part by reduction in plasma volume and a concomitant diminution of tissue fluid.

KURLAND

**Morris, G. C., Jr., Cooley, D. A., Crawford, E. S., Berry, W. B., and De Bakey, M. E.: Renal Revascularization for Hypertension.** *Surgery* 48: 95 (July), 1960.

Normal blood pressure was restored in 82 per cent of 60 hypertensive patients who underwent unilateral or bilateral renal revascularization procedures. In the majority of cases, renal artery bypass was used and, in a few, direct reconstruction of a narrowed segment of the renal artery was carried out with a patch graft. Among the patients in whom the blood flow to the involved kidney was measured, all but one exceeded 200 ml. per minute. Even in patients with extreme bilateral artery stenosis, total blood flow was only minimally depressed. This did not seem to correlate well with the depressed glomerular filtration rate. Among patients with unilateral stenosis, where hypertension had not been of long duration, the uninvolved kidney usually functioned well before operation, while afterwards there was a fall in function below that of the revascularized kidney. This seemed related to a reduced filtration pressure in combination with the nephrosclerotic changes in the uninvolved kidney. In general, the revascularized side showed continued improvement and the uninvolved kidney showed late secondary improvement. The latter indicated some reversibility of the nephrosclerotic

process concurrent with the cure of hypertension. Therefore, the authors suggest that contralateral nephrectomy be delayed for several months to assess the degree of improvement which may occur in the uninvolved side, when this kidney shows severe depression of renal function. In a few patients, pulse wave tracings were obtained before and after revascularization. These showed variable alterations in the contour and amplitude of the pulse pressure wave. Following revascularization, the renal artery pulse waves were indistinguishable from aortic pulse waves. These data, in addition to the above information on renal blood flow before and after revascularization, corroborated experimental evidence indicating that renal ischemia was not the trigger mechanism in the production of renal vascular hypertension and concurred with the current concept that renal hypertension is probably initiated by an alteration in pulse pressure wave. These patients became normotensive after operation and remained so for a maximum follow-up period of 2½ years. The only death was from a coronary occlusion 5 days postoperatively. Severe hypertension without obvious cause warrants renal arteriography, which is the only method of establishing the diagnosis of renal vascular hypertension.

SHEPS

**Oldham, P. D., Pickering, G., Roberts, J. A. F., and Sowry, G. S. C.: The Nature of Essential Hypertension.** *Lancet* 1: 1085 (May 21), 1960.

Opinion is divided between two views of the nature of essential hypertension. The older view is that essential hypertension is a specific disease entity and that subjects with and without the disease can be separated sharply on the basis of their arterial pressures. Hypertension is the expression of a single gene behaving as a mendelian dominant. The new view, presented by the authors, is that essential hypertension represents a quantitative and not a qualitative deviation from the norm with no natural dividing line between normal and abnormal pressures. In this hypothesis the arterial pressure is inherited polygenically over the whole range and the inheritance of the same kind and degree in the normal range as in that characteristic of essential hypertension. The evidence for the older thesis centers on occurrence of the disease in three generations, equality of numbers of affected and unaffected sibs, the rate of rise of pressure with age, and the problem of gene frequency. The authors review existing data relevant to each of these arguments and find them inconsistent, with single-gene inheritance and more in favor of a multifactorial inheritance, arterial pressure being in-

herited as a graded character over the whole range of normal and hypertension.

KURLAND

**Papacosta, C. A., Sevy, R. W. Ohler, E. A., Bellow, C. T., and Brenes, L.: Plasma 17-hydroxycorticosteroid Levels in Normotensive and Hypertensive Subjects and the Influence of Posture.** Am. J. M. Sc. 239: 745 (June), 1960.

Plasma 17-hydroxyeortieosteroid levels in normotensive and treated and untreated essential hypertensive subjects were estimated at rest and under various conditions of stress. There were no significant differences under the conditions of the experiment. It was observed, however, that a significant decline occurred when the subjects were supine for an hour and a half. The authors suggest that consideration should therefore be given to positional change in the interpretation of data dealing with plasma 17-hydroxyeortieosteroids in human subjects.

SHEPS

**Papper, S., Belsky, J. L., and Bleifer, K. H.: The Response to the Administration of an Isotonic Sodium Chloride-Lactate Solution in Patients with Essential Hypertension.** J. Clin. Invest. 39: 876 (June), 1960.

A sodium chloride-lactate solution was administered intravenously to hypertensive and normotensive individuals under rigidly controlled conditions and at three different levels of dietary salt ingestion. The patients with hypertension excreted the infused sodium load more rapidly than normotensive patients at each of the three levels of sodium ingestion. At the low-salt intake level, there was no difference in the quantity of sodium excreted between normal and hypertensive subjects. The exaggerated sodium excretion in the hypertensive patients was not due to differences in preinfusion rates of sodium excretion or to a greater increase in serum sodium concentration. The authors concluded that the observed response to salt administration in the hypertensive patients was still unexplained.

KARPMAN

**Perera, G. A.: Antihypertensive Drug Versus Symptomatic Treatment in Primary Hypertension.** J. A. M. A. 173: 11 (May 7), 1960.

A study was made to determine the possible role of antihypertensive drugs in lengthening the life of patients with primary hypertension. Fifty-eight patients satisfied rigid criteria for the diagnosis of primary hypertension of at least 5 years' duration. They were divided into two matched groups of 29 patients each. The test group re-

ceived a variety of antihypertensive drugs to maintain pressures at or below 160/104 mm. Hg. No such drugs were given to the contra-test group. Both groups received sedatives, analgesics, digitalis, and diuretics as needed. During the period of observation, 16 patients in each group died, and no significant difference in their average survival was found.

KITCHELL

**Tareev, E. M., and Priss, I. S.: Some Disturbances in Lipid Metabolism in Hypertensive Disease.** Cor et Vasa 1: 3, 1959.

In 35 normal persons the fasting serum cholesterol (method of Fedorova) was 125 to 180 mg. per cent, its esterified component 80 to 122 mg. per cent, its free component 45 to 70 mg. per cent, the phospholipids 150 to 240 mg. per cent, and the iodine number 340 to 420 units. Of the 155 patients with hypertension studied, 20 patients with uncomplicated functional hypertension showed normal but widely fluctuating values, while 40 patients in the uncomplicated sclerotic stage showed a slight and persistent elevation of cholesterol, mainly in its esterified component, and often a decreased phospholipid level and a tendency to a decreased iodine number (increased proportion of saturated fatty acids). In 55 patients suffering from complications (hypertensive crisis or myocardial infarction) the phospholipids and the iodine number tended to be elevated during complications with a mild course and decreased during those with a severe course.

LEPESCHKIN

**Tobian, L., Severeike, O., and Cich, J.: Do Mitochondria Participate in General Cardiac Hypertrophy.** Proc. Soc. Exp. Biol. & Med. 103: 774 (Apr.), 1960.

The purpose of this study was to determine how much of an adaptive increase of mitochondrial mass occurred in a typical mammalian muscle that had undergone hypertrophy to meet an increased work load. The hypertrophied wall of the left ventricle of rats (following induced hypertension) was used as a model. Two groups of rats were studied. The control group had blood pressures ranging from 89 to 136 mm. Hg. The other group had one of their renal arteries narrowed with a silver clip. Within 6 months 14 of the rats with narrowed renal arteries had developed moderate to severe hypertension. Arterial pressures averaged 196 for hypertensive rats and 119 for normotensive rats. After the rats were killed, the left ventricular wall and interventricular septum were removed, weighed, and cut into small cubes and then suspended in various solu-

tions and centrifuged. The following results and conclusions were drawn. Left ventricular weight: body weight ratio was 55 per cent greater in hypertensive rats than in a similar group of normotensive animals. The ratio of "non-particulate" proteins of the left ventricle to total body weight was 53 per cent greater in the hypertensive group, mitochondria of the left ventricle also participated in the cardiac hypertrophy of the hypertensive group. Submitochondrial particles of the left ventricle also participated in cardiac hypertrophy, and the ratio of protein in these particles to total body weight was 37 per cent greater in hypertensive rats than in normotensive rats. Hypertrophy of the mitochondria, however, was not as great proportionately as that of the left ventricle as a whole. Hypertrophy of the submitochondrial particles in hypertensive rats was proportionately less than the hypertrophy of the left ventricle as a whole. The mass of protein individual mitochondrion was slightly less in hypertensive rats than in the normotensive animals. The total number of mitochondria increased 50 per cent as the left ventricle hypertrophied ( $p=.08$ ). It seemed evident that the total mass of mitochondrial protein in the left ventricle could increase in adaptation to an increase in work load.

KRAUSE

### METABOLIC EFFECTS ON CIRCULATION

Luthy, E., Rosli, R., and Bischof, B.: Behavior of Cardiac Glycogen in Hypertrophied Rat Hearts after ACTH and STH. *Cardiologia* 36: 209, 1960.

Left heart hypertrophy was produced in rats in a short period by establishing coarctation of the abdominal aorta. During development of this hypertrophy the glycogen content of rat hearts was increased. The glycogen level depended on the rate of development of hypertrophy. ACTH or STH produced an increase in heart glycogen, followed by a fall. In the hypertrophied heart this increase was much less than in normal animals but the fall was greater. Possible mechanisms are discussed.

BRACHFELD

### PATHOLOGY

Ghys, A., and Vastesaeger, M.: Coronarographic Aspects of Coronary Occlusions. *Acta cardiol.* 15: 120, 1960.

Experience with 360 postmortem arteriograms of the coronary arteries, of which 200 were in cases of myocardial infarction, are discussed. The injection was performed according to the classic

technic as described by P. Vander Spraeten. It was concluded that these studies facilitate the diagnosis of the cause of a coronary occlusion, particularly recent thrombosis. However, as the thrombus retracts and organizes, the radiologic picture becomes less characteristic. If the occlusion is old, the picture is often irregular and its interpretation difficult, particularly if the lesion is in the process of recanalization and if vascular calcification has occurred. Increased information is obtained by combining coronary arteriography with a dissection of significant areas of the coronary network.

BRACHFELD

Gore, I., and Collins, D. P.: Spontaneous Atheromatous Embolization. Review of the Literature and a Report of 16 Additional Cases. *Am. J. Clin. Path.* 33: 416 (May), 1960.

Embolization of atheromatous material has occurred with sufficient frequency to make it significant. It may occur spontaneously or as the result of surgical manipulation of a diseased artery. Most of these emboli are recognized microscopically. Their structure is identical to the contents of degenerating atheromatous lesions of the aortic intima. Lack of attachment to a structurally intact wall of a vessel and the presence of intact erythrocytes at the perimeter of the embolus characterize the fresh lesion. Since most of these emboli occur in small vessels, endogenous atherosclerosis of these vessels can be excluded by the presence of crystalline lipid deposits. To date 84 cases have been described; 66 occurred spontaneously and 18 complicated surgical resections of abdominal aortic aneurysms producing renal insufficiency and death. The presence of an aneurysm increases significantly the incidence of atheromatous embolization. The present report describes the detailed findings in 16 patients. The most commonly involved viscera were the kidney, pancreas, and spleen. This is probably explained by the greater involvement of the abdominal aorta which supplies these viscera. These 16 cases were accumulated in a few months indicating the frequency of this complication, particularly after the age of 60. It was noted that the lesions were frequently multiple and of varying ages. Cerebral emboli commonly originated from plaques in the carotid arteries. Patients with cerebral or coronary emboli had a high incidence of syphilis, accounting for unusually advanced intimal disease in the proximal aorta. Pancreatitis and gastrointestinal ulcers have been reported as a result of atheromatous emboli. The aorta was the principal source of origin for the embolic material.

LEVINSON

**Hudson, R. E. B.: The Human Pacemaker and Its Pathology.** *Brit. Heart J.* 22: 153 (Apr.), 1960.

The sinoatrial node was examined histologically in 65 formalin-fixed human hearts, including one fetal heart. All except the fetal heart were abnormal in some respect. The landmark for the normal node is the summit of the junction of the superior vena cava and the right atrium. The average normal adult node is a crescentic vascular neuromyoendocardial structure distinctly marked off from the neighboring tissues. At its largest part, it is three to four times the size of a pin head. In 14 of 15 patients with obviously damaged nodes, cardiac arrhythmias had been present in life. Two patients showed evidence of additional surgical trauma following operations for correction of atrial septal defects. The commonest of all node lesions in this series was focal hemorrhage; hypertrophy and neoplastic involvement occurred uncommonly.

KALMANSONH

**Huntingford, P. J.: The Aetiology and Significance of Congenital Heart Block. (The Report of a Case Studied by Serial Section of the Heart.)** *J. Obst. & Gynaec. Brit. Emp.* 67: 259 (Apr.), 1960.

The author described the eighth patient with congenital heart block in whom serial sections of the heart were studied. The patient was a 3-day-old girl who died with a heart rate of 60 per minute and cyanosis. The atrioventricular node was not identified. The first identifiable conducting tissue was a bundle running vertically in the central fibrous body which separated the atrial and ventricular musculature. As it coursed through the central fibrous body, the bundle became split into disconnected strands of loose fibrous tissue. The remainder of the sections were apparently normal except for the presence of a defect in the membranous interventricular septum. By reviewing the other seven patients subjected to histologic studies of the conducting system the author concluded that congenital heart block may occur in many types of congenital heart disease and in otherwise normal hearts, that an interventricular septal defect per se is not the cause of the block, and that the condition results from defective formation of the central fibrous body.

KALMANSONH

**Larsen, K. A., and Noer, T.: Cardiac Aneurysm of the Membranous Portion of the Interventricular Septum.** *Acta med. Scandinav.* 166: 401, 1960.

Three cases of aneurysm of the membranous portion of the interventricular septum are described. The first patient was a woman aged 72 who had experienced various types of arrhythmias and died in heart failure. There was no constant murmur. A second woman aged 70, died in heart failure with no clinically recognizable symptoms attributable to the aneurysm. The final patient was a woman aged 45 who developed hypertension due to chronic pyelonephritis. A rough systolic murmur was subsequently heard at the second left interspace, and a late systolic, thin, sibilant murmur was also heard. It was postulated that the latter murmur was caused by an increase in tension within the aneurysm secondary to the hypertension; this possibly affected the tricuspid orifice during systole producing regurgitation. These aneurysms always protrude into the right heart, either above, at, or below the tricuspid valve. It is suggested that because of the location of these aneurysms, they may cause symptoms by interfering with the tricuspid valve in late systole, or may be the cause of some rhythm disturbances affecting the bundle of His-Tawara. Other cases in the literature with arrhythmias are noted. This condition appears to be due to congenital maldevelopment and commonly is seen associated with Mongoloidism.

SHEPS

**Lurie, A. O.: Left Ventricular Aneurysm in the African.** *Brit. Heart J.* 22: 181 (Apr.), 1960.

Because of the low incidence of ventricular aneurysm in the African and the heretofore unrecorded incidence of myocardial infarction as the etiology of these rare patients with ventricular aneurysm, the author reports four patients with this condition, in three of whom postmortem examinations were performed. In three patients, no etiology for the aneurysm was discovered; the fourth patient constitutes the first African to be reported with a ventricular aneurysm due to coronary thrombosis; the atypical features of the latter patient were the age (31-year-old man) and the absence of coronary artery atherosclerosis or other lesions that could be held responsible for the formation of a thrombus.

KALMANSONH

## NEWS FROM THE AMERICAN HEART ASSOCIATION

44 East 23rd Street, New York 10, New York  
Telephone Gramercy 7-9170

### September 15 is Deadline to Apply For AHA Fellowship Awards

Applications for *Research Fellowships* and *Established Investigatorships* to be awarded by the Association for the fiscal year beginning July 1, 1962, should be submitted by September 15, 1961. The deadline for Grants-in-Aid applications is November 1, 1961.

Fellowship awards and their range of stipends are: *Established Investigatorships*, \$7500-\$9900, plus special grant and dependency allowances; *Advanced Research Fellowships*, \$5500-\$6000, plus departmental and dependency allowances; *Research Fellowships*, \$4500-\$5000 plus dependency allowances (this category is awarded primarily by local Heart Associations).

Forms for submitting applications are available from the Associate Medical Director for Research, American Heart Association, 44 East 23rd Street, New York 10, New York.

### Record Number of Abstracts Submitted For AHA Scientific Sessions

A record number of 627 abstracts have been submitted for possible presentation at the 34th annual Scientific Sessions of the American Heart Association. The sessions will be held at the Americana Hotel in Bal Harbour, Miami Beach, Florida, October 20-22.

The program will include six sessions on clinical cardiology designed particularly for the practicing physician. A panel or symposium on related investigative work will be presented at each clinical session.

Also, a total of at least 18 other scientific

sessions will be held concurrently throughout the three-day program.

Following is an outline of the program:

#### Friday, October 20

Opening address by Oglesby Paul, M.D., AHA President; Conner Memorial Lecture, by Dr. Clark H. Millikan, Professor of Neurology, Mayo Clinic; symposia, "Contribution of Phonocardiography to Auscultation," and "Coronary Arteriography"; lecture, "Biplane Angiography" by Dr. Herbert L. Abrams, Assistant Professor of Radiology, Stanford Medical Center; concurrent sessions on various cardiovascular subjects; and a program for nurses.

#### Saturday, October 21

Panel on "Ventricular Arrhythmias"; lecture on "Closed Chest Cardiac Resuscitation" by James R. Jude, M.D., Johns Hopkins Hospital; Brown Memorial Lecture, "Physiology of the Peripheral Circulation," by Robert W. Wilkins, M.D., Professor of Medicine, Boston University School of Medicine; symposium, "Renal Failure"; simultaneous sessions on basic science, cardiovascular surgery, and "Compensable Heart Disease, Strain and Trauma."

Conferences on a variety of cardiovascular topics will be held on Saturday evening.

#### Sunday, October 22

Symposium, "The Role of Hormones in Heart Failure"; panels, "Ventricular Hypertrophy and Bundle Branch Block" and "Newer Electrocardiographic Lead Systems"; lecture, "ECG Clues Suggesting Myocardial Infarction" by Junior A. Abildskov, M.D., Assistant Professor of Medicine, State University of New York College of Medicine; and

concurrent sessions on rheumatic fever and congenital heart disease and cardiovascular surgery. Cardiovascular films, with introductions and commentary by the author or other authority on the subject, will be shown throughout Sunday.

#### AHA to Issue "Circulation Research" As a Monthly Beginning in 1962

To accommodate an increasing volume of research papers on basic studies in the cardiovascular field, *Circulation Research* will be issued monthly instead of bi-monthly, beginning January, 1962.

The change reflects the steady expansion of research which has been taking place in the cardiovascular field during recent years. *Circulation Research* was established by the Association in 1953 as a 96-page publication, and has since been enlarged to its present 256-page size. This has not been sufficient, however, to keep pace with the growing volume of worthwhile manuscripts received. Publication of *Circulation Research* on a monthly basis will help speed the transmission of research findings to investigators.

One of three scientific periodicals published by the American Heart Association, *Circulation Research* is the only journal devoted exclusively to reports on fundamental research studies as they apply to cardiovascular medicine and surgery.

Subscription rates for the journal as a monthly will be \$14 annually in the U.S. and Canada, \$15 elsewhere. It will be available at a reduced rate of \$9 for full-time research fellows, interns, residents and medical students. A combined subscription to *Circulation Research* and *Circulation* will be \$25 yearly, \$28 outside the U.S. and Canada.

#### Materials from AHA Journals Are Available in Volume Form

Four volumes bringing together materials of interest to physicians and scientists have been published by the Association, as follows:

*Hypertension-Chemical and Hormonal Factors*, Volume IX of the Proceedings of the Association's Council on High Blood Pressure

Research, a compilation of papers given by leading authorities in the field at the Council's Annual Meeting in Cleveland last November (\$2.50), printed as a supplement to *Circulation Research*, May 1961.

*Symposium on Coronary Heart Disease*, No. 2 in the AHA monograph series, a compilation of articles originally published in *Circulation* from August 1960-January 1961 (\$2.50);

*Cardiovascular Abstracts I—1960* contains more than 700 abstracts of significant papers on cardiovascular subjects published in 86 scientific journals in the U. S. and abroad (\$2.75). These originally appeared in sections of *Circulation*.

*The Myocardium—Its Biochemistry and Biophysics* includes presentations given at a New York Heart Association symposium in December 1960 (\$2.50), also published as a supplement to this issue (August) of *Circulation*.

Copies of all volumes are available from the Distribution Department, American Heart Association, 44 East 23rd Street, New York 10, New York, through local Heart Associations or through medical bookstores.

#### Article on Heart Auscultation Available to Physicians, Students

To help meet the need for continuing training and education in auscultation of the heart, the Association has made available to physicians and medical students reprints of an article on the subject which appeared in the March and April, 1961, issues of *Modern Concepts of Cardiovascular Disease*.

The article, by Virginia Hardman, M.D. and J. Scott Butterworth, M.D., of New York University School of Medicine, discusses briefly some of the physical and hemodynamic principles which govern production of heart sounds and murmurs. It points out some factors which influence their recognition under clinical circumstances. Included are schematic diagrams and a list of reference books.

Copies are obtainable free from local Heart Associations or the American Heart Association.

**Delaware Heart Group Donates \$12,250 To Supplement AHA National Research**

The Delaware Heart Association has provided \$12,250 to supplement the Association's national research program for fiscal 1961-62. The funds will be applied to support of the following Grants-in-Aid:

Helen B. Taussig, M.D., Johns Hopkins University, in full support of a study on "Etiology of Congenital Malformations of the Heart and Great Vessels"; Francis Wood, M.D. and Earl Barker, M.D., University of Pennsylvania School of Medicine, in full support of a study on "Renal Physiology—Normal and Pathologic"; also, Dr. Wood and Stanley A. Briller, M.D., for total support of a study on "Energetics of the Myocardium"; and Emil Blair, M.D., University of Maryland School of Medicine, in partial support of a grant for "Physiological, Morphological and Surgical Study of Experimental Coronary Thrombosis and Myocardial Infarction."

Such contributions over amounts regularly assigned by local Heart Associations for national research permit support of additional studies which could not otherwise be covered by the national research budget.

**New Heart Bulletin Editors Are Named by Association**

The Association's Publications Committee has announced the appointment of seven physicians as Associate Medical Editors of *The Heart Bulletin*, which is sponsored by AHA in cooperation with the National Heart Institute and the American Academy of General Practice.

The new editors take the places of several previous appointees whose terms have expired. They will advise Dr. Russell W. Cumley, who remains as Executive Editor of the bi-monthly journal.

The newly-named Associate Medical Editors and their fields of specialization are:

David I. Abramson, M.D., Chicago, Peripheral Circulation; Henry T. Bahnsen, M.D., Baltimore, Cardiovascular Surgery; Stanley Briller, M.D., Philadelphia, Electrocardiography; Edward C. Lambert, M.D., Buffalo,

New York, Pediatrics; Milton Mendlowitz, M.D., New York, Hypertension; William L. Proudfoot, M.D., Cleveland, Clinical Cardiology; and Roger W. Sevy, M.D., Philadelphia, Research.

A bi-monthly journal on cardiovascular disease for the practicing physician, *The Heart Bulletin* is published as a public service by the Medical Arts Publishing Foundation, Houston.

**Postgraduate CV Surgery Courses Offered by College of Surgeons**

Because of wide interest in the subject, special attention is called to a series of half-day postgraduate courses on "Cardiovascular Surgery" to be given during the American College of Surgeons' Clinical Congress in Chicago, October 2-6.

Subjects for the courses are "Vascular Surgery," "Hypothermia in Cardiac Surgery," "Certain Problems of Congenital Heart Disease," and "Problems of Extracorporeal Circulation." The fee is \$10 per course. Concurrent general sessions are scheduled on surgery and surgical specialties.

Registrations may be made with the American College of Surgeons, 40 East Erie Street, Chicago 11, Illinois.

**Gift Subscriptions to AHA Journals Urged for Needy Countries**

As an aid to world-wide dissemination of cardiovascular knowledge, physicians and scientists in the United States are urged to donate subscriptions to *Circulation* and *Circulation Research* to medical university libraries, schools and professional groups in underdeveloped and newly independent countries.

Those wishing to offer such gift subscriptions may do so through the Association's Publishing Office, 44 East 23rd Street, New York 10, New York. The cost of each subscription will be: \$15 for *Circulation*, \$10, *Circulation Research*. Subscriptions to the combined journals are \$23.

**Meetings Calendar**

August 27-September 1: American Congress of Physical Medicine and Rehabilitation, Cleveland. Dorothea C. Augustin, 30 N. Michigan, Chicago 2, Illinois.

September 26-29: American Roentgen Ray Society, Miami Beach. C. A. Good, Mayo Clinic, Rochester, Minnesota.

October 2-6: American College of Surgeons, Chicago. W. E. Adams, 40 East Erie St., Chicago 11, Illinois.

October 3-4: Congress on Occupational Health, Denver. Council on Occupational Health, American Medical Association, 535 N. Dearborn, Chicago 10, Illinois.

October 12-14: International College of Surgeons, Regional Meeting, Atlantic City. W. F. James, 1516 Lake Shore Dr., Chicago 10, Illinois.

October 14-20: International Congress of Neurosurgery, Washington, D.C. Bronson S. Ray, 525 E. 68th Street, New York 21, New York.

October 17-19: International Seminar on Vascular Systems, Miami Beach. John B. Liebler, Heart Association of Greater Miami, 253 S.W. 8th St., Miami 36, Florida.

October 18-20: Council on Arteriosclerosis of the American Heart Association, Bal Harbour, Florida. Jeremiah Stamler, Chicago Board of Health, 54 West Hubbard, Chicago 10, Illinois.

October 20-24: American Heart Association, Annual Meeting and Scientific Sessions (October 20-22), Bal Harbour, Florida. American Heart Association, 44 East 23rd St., New York 10, New York.

October 26-27: The Organization of Bio-Medical Instrumentation and Engineering in Universities and Hospitals, Omaha. Office of Medical Extension, University of Nebraska, Omaha 5, Nebraska.

November 13-17: American Public Health Association, Detroit. Berwyn F. Mattison, 1790 Broadway, New York 19, New York.

November 13-18: Canadian Heart Association and National Heart Foundation of Canada, Annual Meeting and Scientific Sessions, Vancouver. J. B. Armstrong, National Heart Foundation of Canada, 501 Yonge St., Toronto 5, Canada.

November 16-18: International Symposium "Etiology of Myocardial Infarction," Detroit. Thomas N. James, Henry Ford Hospital, Detroit 2, Michigan.

November 25-27: American College of Chest Physicians, Interim Session, Denver. W. Bentley, 112 E. Chestnut, Chicago 11, Illinois.

November 27-30: American Medical Association, Clinical Meeting, Denver. F. J. L. Blasingame, 535 N. Dearborn, Chicago 10, Illinois.

December 1-2: Symposium on Cinefluorography (3rd) Rochester, New York. Stanley M. Rogoff, University of Rochester Medical Center, Rochester 20, New York.

**1962**

February 7-10: American College of Radiology, New York. W. C. Stronach, 20 No. Wacker Dr., Chicago 6, Illinois.

**Abroad**

September 3-7: International Congress on Rheumatology, Rome. Prof. C. B. Ballabio, Clinica Medica Generale, Via F. Sforza 35, Milano, Italy.

September 3-10: Inter-American Congress of Radiology, Sao Paulo. W. Bomfim-Pontes, Rue Cesario Motta, No. 112, Sao Paulo, Brazil.

September 4-9: International Congress of Angiology, Prague. Prof. Z. Reinis, IVth Medical Clinie, Praha 2/499, Czechoslovakia.

September 6-12: International Congress of Human Genetics, Rome. Luigi Gedda, 5 Piazza Galeno, Rome, Italy.

September 7-9: International Cardiovascular Society Congress (5th), Dublin. H. Haimovici, 715 Park Ave., New York 21, New York.

September 10-15: International Neurological Congress, Rome. G. Alema, Viale Universita 30, Rome, Italy.

September 11-14: National Congress of Cardiology, San Luis Potosi, Mexico. Jose M. Torre, Av. V. Carranza No. 2405, San Luis Potosi, S. L. P., Mexico.

**1962**

October 7-13: Fourth World Congress of Cardiology, Mexico City. I. Costero, Secretary General, Ave. Cuauhtemoc 300, Mexico 7, D. F.

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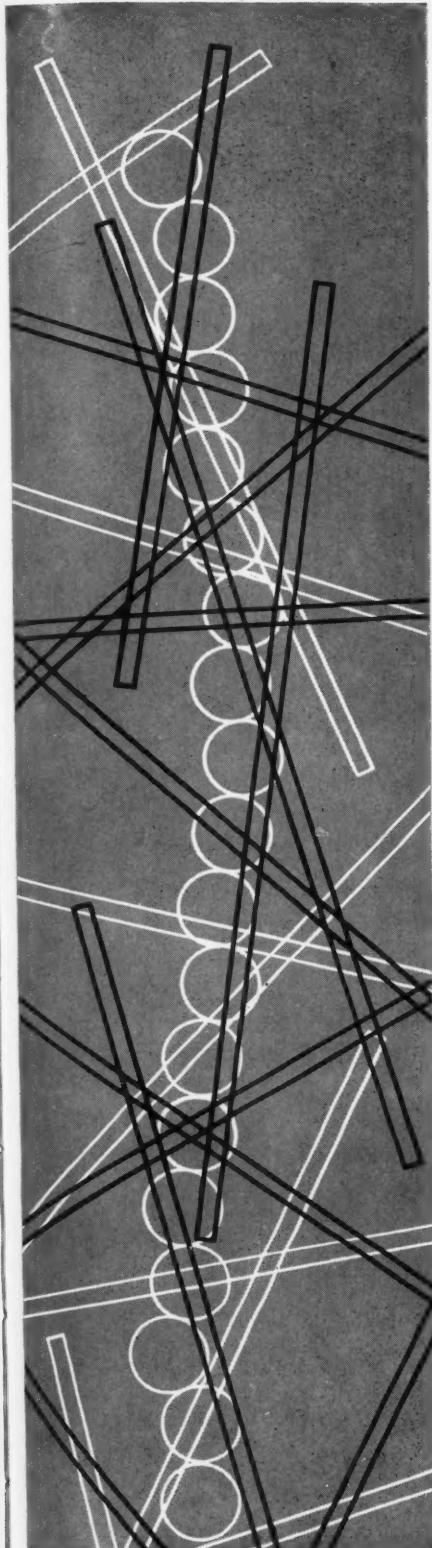
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**THE MYOCARDIUM—  
ITS BIOCHEMISTRY  
and  
BIOPHYSICS**

**NEW YORK HEART ASSOCIATION, INC.**

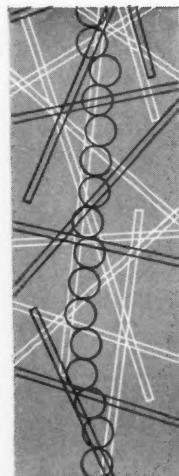
New York City, December 9-10, 1960

**Alfred P. Fishman, M.D.  
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*The symbols  
represent a diagram  
of an "actomyosin swarm"  
by Wilfried F. H. M. Mommaerts*



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\* Deceased May 29, 1960.

# Circulation

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## Foreword

IT IS TRADITIONAL, both in the physiologic laboratory and in the clinic, to regard the heart as a muscular pump. Moreover, in appraising the performance of this pump, it has become customary to assess separately the work of the right and of the left ventricles, a practice that involves the measurement of the blood pressure developed and the volume of blood ejected by each ventricle during systole. From this hemodynamic approach has come not only the broad generalizations about the behavior of the heart, i.e., "Starling's Law," "Bowditch's All or None Law" and the "Staircase Phenomenon," but also the criteria for estimating the capacity of each ventricle for performing work, as well as its adaptability to different work loads.

However, as it becomes clear that current hemodynamic measurements *per se* promise fewer and fewer broad generalizations, attention is turning to the biochemical and biophysical aspects of heart muscle. The heart is being scrutinized as a biologic engine for transducing the chemical energy of metabolism into useful mechanical work. Leading and directing this new approach are the electron microscopists, the biochemists, and the biophysicists who bring lessons learned from muscular and nonmuscular contractile tissues to the study of the myocardium.

Their first studies have succeeded in illuminating certain features of myocardial function. But, these studies have also emphasized how little we know about the coordinated

performance of the heart. Indeed, in the sphere of coordinated behavior, they have raised more questions than they have answered: How does the myocardial engine work? How is the chemical energy converted into mechanical energy? Is there a general principle of contractility and how does the myocardium illustrate it? What sets the contractile process into motion? What is the molecular basis for electric-chemical-mechanical coupling?

These questions about the coordinated behavior of the heart muscle should not obscure the wealth of observations and experiments that preceded them. Muscle has probably been studied more intensively than any other tissue. Indeed, such questions could not be posed were it not for a host of antecedent observations and experiments. Unfortunately, only the future can disclose if the proper observations were made, if the proper experiments were performed, and if the proper questions are being asked.

Although this conference is deeply rooted in classical physiology, nearly all of its topics are products of modern times. This point is easily illustrated with respect to the energy for muscular contraction. Thus, only in 1907, did Fletcher and Hopkins disclose that lactic acid is formed when muscle contracts. By the 1920s, the time was ripe for Hill, Myerhof, and Fenn to consider, in a systematic fashion, the energy liberated by a contracting muscle. By 1930, it was clear that: (1) myosin is part of the contractile machinery of muscle,

(2) the energy expended during contraction derives from the breakdown of glycogen into lactic acid, and (3) creatine phosphate and ATP are present in muscle. During the 1930s, the role of creatine phosphate was elucidated and parallels were drawn between glycolysis in muscle and alcoholic fermentation. Not until the 1940s was it shown that: (1) myosin catalyzed the splitting of ATP, (2) actin and myosin were separable, and (3) the contractile behavior of muscle could be mimicked *in vitro*. In the same decade, the electron microscope was also first applied to the visualization of the ultrastructure of heart muscle. In the 1950s and 1960s, attention has been focused on the intracellular, molecular sources of energy for contraction and on models that are designed to account for the behavior of contracting muscle *in vivo*.

This outline of a steady progression from larger to smaller units has skipped the uncertainties that haunt the isolation of cellular ingredients from their natural surroundings. Is the final preparation typical of living muscle or is it an artifact of synthesis? What does the behavior of the contractile thread or the isolated sarcomere mean for the behavior of intact muscle? Mephistopheles recognized that "to comprehend a living thing past

any doubts" it is necessary to "cancel first the living spirit out." But, how does the final analysis take into account the "living link you banned"? How relevant is the behavior of skeletal or smooth muscle to the behavior of heart muscle? Is there a general law of contractility that is epitomized by the behavior of heart muscle? How meaningful are the ingenious anatomic and physicochemical models for the understanding of living muscle?

The time is not yet ripe for answers to many or, possibly, to any of these questions. Nor was this meeting arranged to provide such answers. Instead, the meeting has more circumscribed goals: to set questions that await solution alongside of the observations that have led to their formulation; to encourage investigators to illuminate boundaries between different interests so that these boundaries may be crossed; to allow the uninitiated to peer at muscle through the disciplined eyes of scientists devoted to the study of muscle; and, hopefully, to help to shape a question or two that might otherwise have remained amorphous.

ALFRED P. FISHMAN, M.D.  
*Guest Editor*

### Muscle as a Contractile Tissue

... it must not be supposed that muscles operate by any unique mechanism not represented in other contractile tissues. The fundamental mechanism of contraction is presumably the same in all tissues, but in muscles it is less obscured by other functions, such as digestion, absorption and excretion, and it is easier to measure the forces developed, easier to observe the physical changes which occur, and easier to determine the chemical nature and quantities of the reactants and end products of the chemical processes involved.—W. O. Fenn. *Section on Contractility* in R. Höber, *Physical Chemistry of Cell and Tissues*. Philadelphia, Blakiston, 1945, p. 447.

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## I. Ultrastructure

Chairman: Charles E. Kossmann, M.D.

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### On Smallness

By CHARLES E. KOSSMANN, M.D.

BY WAY OF INTRODUCTION to the material on Ultrastructure to be presented in this initial portion of the Symposium on the Myocardium a few remarks on the general problem of Smallness are in order.

When one probes a little into the scientific realm of the previously unseen, some paradoxes and apparent inconsistencies are encountered that make comprehension by the uninitiated a little difficult. I will attempt to share with you 1 or 2 of the personal problems encountered in learning about this strange new world, which I hope may implement your own understanding of the exciting presentations about to be made.

We might begin with the word, Ultrastructure, itself. "Ultra" is a prefix that, in the Latin from which it is borrowed, means "beyond." "Beyond structure" by itself is relatively meaningless, but perhaps those who coined the word had in mind a structure beyond something. That something is visibility, and, for purposes of definition, visibility even when augmented with a compound light microscope.

Ultrastructure, then, is really a contraction of "ultramicroscopic structure" meaning that it is substance of such small size as to be beyond that which can be seen with a light microscope. Despite the definition, a microscope is indeed used. But it is a special type, available for barely 2 decades but nevertheless now quite familiar to most morphologists. It is the electron microscope.<sup>1</sup>

Human curiosity naturally prompts one to

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New York, New York.

ask how the electron microscope differs from an ordinary light microscope. Both utilize the refractive principle but in one the refracted beam is light, in the other a stream of electrons. When the mechanisms involved are investigated further, a singularly surprising discovery is made to the effect that, in the field of electron optics, a dualism exists which on the surface, at least, appears to be a paradox. The electron is regarded not only as a particle, but also as a *wave*. Further, and most important from the microscopist's point of view, the wave has a length that is only a fraction of the length of an ordinary light wave. Basically it is this difference in wave lengths that accounts for the different resolving powers of the 2 types of microscopes under discussion. For the light microscope the limit of resolution for tissues is in the neighborhood of 0.25 micron; of an electron microscope 20 to 30 Å. Under ideal conditions, then, the resolving power of the latter is 100 times greater than of the former. In the biologic sciences it is the new anatomic realm revealed by this superior performance of the electron microscope that constitutes what is now known as Ultrastructure.

It might be appropriate at this point to recall the measurements used in defining degrees of smallness. Justification for repeating these elementary measurements is the presumption that most of you do not use the electron microscope in your everyday activities.

$$1 \text{ mm.} = 1,000 \mu = 10,000,000 \text{ \AA}$$

Another way of saying this is that

$$0.001 \text{ mm.} = \mu = 10,000 \text{ \AA}$$

By way of a minor but purposeful digression, if the problems of wave mechanics and theory as applied to the electron microscope are studied, it is learned that the electrons in the beam, being particles, have energy defined by Einstein's well-known relation for the equivalence of mass and energy, namely

$$E = mc^2$$

The equation has relevance to more than a beam of electrons. To the physicist it demonstrates, among other things, the important concept of variation of mass with velocity. To the biologist it suggests, if it is correct, that eventually the division between structure and function must become narrow indeed. As this symposium wends on, all of us will probably become aware of the considerable decrease in the gap between structure and function that has resulted from the original investigations about to be presented.

In considering the degrees of smallness with which our distinguished speakers on Ultra-

structure have concerned themselves, one wonders what will be the next order of magnitude to be studied. It is obvious, for example, that pores exist in membranes which cannot be seen even with the electron microscope.<sup>2</sup> What will be the eventual extreme of visible smallness? How small is infinitely small? What is the limit of smallness of which man's mind can conceive? In such frustrating questions as these can be recognized the substrate for a new type of neurosis among ultra-microscopists and perhaps among all biologists. But this theoretical occupational disease need not concern us at present. There is too much yet to be learned about ultrastructure now visible, and it is about this as found in the myocardium that we will hear this morning.

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### One Life

Four decades of research have left no doubt in the author's mind that there is only one life and one living matter, however different its structures, colorful its functions, and varied its appearance. We are all but recent leaves on the same old tree of life and even if this life has adapted itself to new functions and conditions, it uses the same old basic principles over and over again. There is no real difference between the grass and he who mows it. The muscles which move the mower need the very same two substances for their motion as the grass needs for its growth, potassium and phosphate, the two substances we put on our lawn as fertilizer so as to have something to mow—a strikingly simple demonstration of the basic unity of living Nature,—A. Szent-Györgyi. *Chemistry of Muscular Contraction*. Ed. 2. New York, Academic Press Inc., 1951, p. 5.

# The Contractile Structure of Cardiac and Skeletal Muscle

By H. E. HUXLEY, PH.D.

Previous findings that have led to the 'sliding-filament' model for striated muscle are reviewed, together with some recent observations on isolated filaments produced by a new procedure. The basic structure of the contractile apparatus in skeletal and in cardiac muscle appears to be identical. The relation between certain special features of cardiac muscle and its structure is discussed.

I SHOULD LIKE first of all to review very briefly the picture we now have of the fine structure of striated muscle. This will be very familiar to many people here, but I hope to mention one or two new pieces of evidence to maintain their interest. It will be useful to set out this picture again, I believe, since many of its features are established with a rather high degree of certainty and have been accepted by its original opponents; it is therefore likely to be both profitable, and fairly correct to think of muscles in these terms when trying to explain their various properties. The features I shall describe appear to be common to both cardiac and skeletal muscle.

## Review of Structural Observations

The starting point in describing the fine structure is the well-known appearance of the cross striations in these muscles, illustrated in figure 1. This shows the characteristic alternation of dark and light bands along each of the myofibrils, the dense A-bands, and the less dense I-bands. The I-bands are bisected by the dense Z-line, and the A-bands often show a less dense zone in the center, known as the H-zone. The myofibrils are also composed of longitudinal filaments, and, when very thin sections are examined (fig. 2), it becomes evident that it is the arrangement of the filaments that gives rise to the pattern of striations, as Dr. Jean Hanson and I suggested on the basis of light-microscope observations and the earlier electron micrographs.<sup>1</sup> There are

2 types of filaments present, organized into a series of overlapping arrays along each fibril, arrays of thicker filaments forming the A-bands and arrays of thinner filaments being present in the I-bands. The thinner filaments extend into the A-bands but at rest length do not quite reach to the center, leaving there, as a result, the somewhat less dense H-zone. The thick filaments have a diameter of about 100 Å and lie in a hexagonal array about 450 Å apart; they are each about 1.5 microns long, the length of the A-band. The thin filaments are about 50 Å in diameter and extend approximately 1 micron on either side of the Z-line. At resting length, each sarcomere (Z to Z) is about 2.3 microns long, so the width of the H-zone is approximately 0.3 micron.

Cross-bridges extend between the thick and thin filaments. Each thin filament is connected to each of its 3 neighboring thick filaments by a bridge every 400 Å along the length of the overlap region, giving it a total of about 54 bridges at resting length. The total number of bridges in 1 cc. of muscle is of the order of  $5 \times 10^{16}$ . It is very plausible to suggest that the bridges provide a means by which chemical and mechanical interaction can take place between the arrays of filaments.

Muscle fibrils can be disintegrated mechanically in the presence of agents that weaken the forces of attachment of the cross-bridges. When this is done, the structure breaks down into (1) isolated thick (100 Å diameter) filaments, nearly always 1.5 microns in length, showing projections reminiscent of the cross-bridges seen in intact muscle;

From the Biophysics Department, University College, London, England.

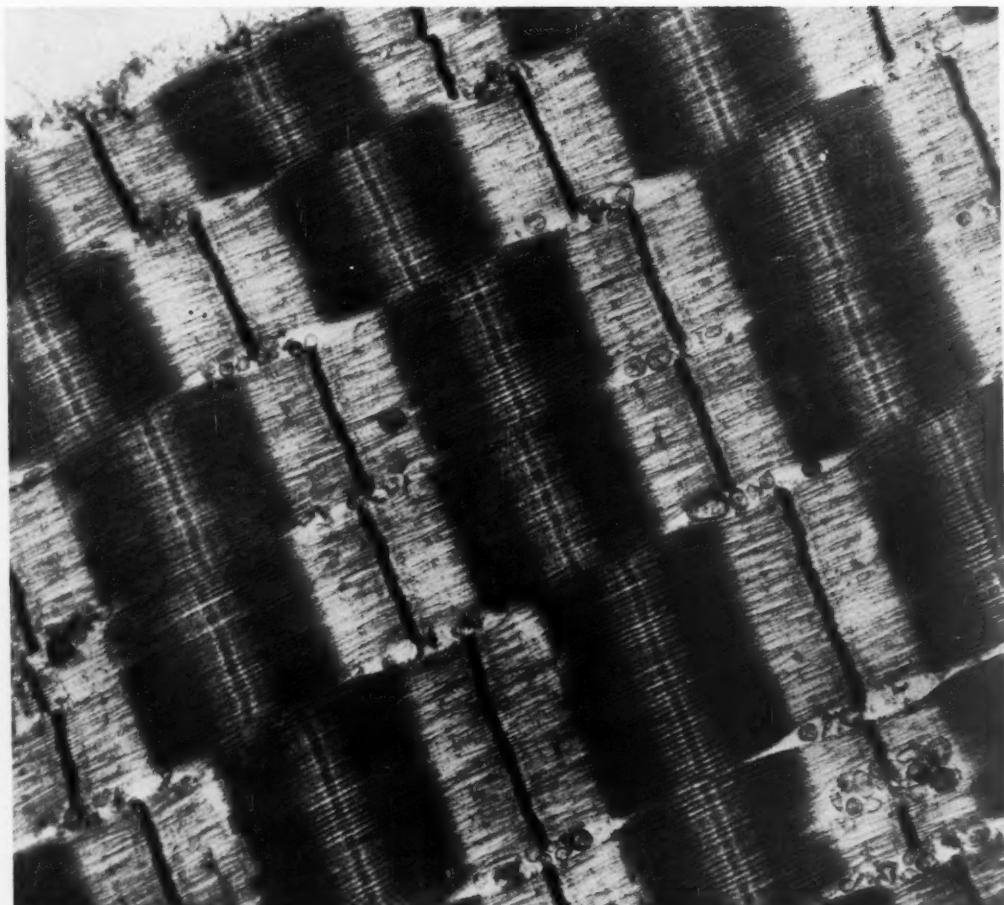


Figure 1

*Low magnification electron micrograph of thicker section of rabbit psoas muscle showing a number of myofibrils near the edge of a fiber. A-band dense; I-bands less dense and bisected by Z-line.  $\times 27,000$ .*

(2) isolated thin (50 Å diameter) filaments, sometimes 1 micron long and sometimes apparently broken into smaller lengths; (3) groups of thin filaments still joined onto a Z-line, forming an "I-segment" about 2.0 microns in length; and (4) occasional groups of thin and thick filaments lying side by side and seemingly joined together by cross-bridges. These various structures are illustrated in figures 3, 4, and 5; their appearance provides a new form of confirmation of our previous con-

clusions. They also provide a new type of experimental material, both for electron microscopy and perhaps for biochemical studies also.

Now let us consider the composition of the filaments. At present there are very strong reasons for believing that the thick filaments contain all the protein myosin that is present in the muscle, while the second principal structural protein, actin, occurs in the thin filaments. Solutions known to dissolve out myosin from the muscle selectively



Figure 2

*High magnification picture of thin section of rabbit psoas muscle showing arrangement of thick and thin filaments.  $\times 150,000$ .*

will dissolve out the thick filaments,<sup>1,2</sup> leaving the array of thin filaments behind, a process that can be observed in the light microscope as the disappearance of the dense A-bands. Subsequent extraction of actin dissolves away most of the material of the thin filaments. These observations have been put on a quantitative basis by the use of the interference microscope.<sup>3,4</sup> The amount of material in the A-bands, arising from the presence of the thick filaments, very closely approximates the amount of myosin present, and the amount of material removed from the A-bands is quantitatively equal to the amount of myosin that could be extracted from the same type of preparation by large-scale biochemical technics. More recently, Perry and Corsi<sup>5</sup> have shown that the selective removal of actin and tropomyosin removes the I-band material, leaving the A-bands (and all the ATPase activity and hence presumably the myosin) intact.

The changes that take place in this

structure during changes in the length of the muscle can be investigated by light-microscope observations as changes in band-pattern. These show<sup>6,7</sup> that during stretch and during shortening, active or passive, the two sets of filaments slide past each other, there being no substantial overall change in the length of any of the filaments until it is brought about by steric factors in more pronounced degrees of shortening (e.g., when sarcomere length decreases below the length of the A-bands). When the actin filaments have come together in the center of the sarcomere, further shortening seems to cause them to slide past each other, giving the double overlap effect illustrated in figure 6.

These observations, and others, lead us to think that the system must function in the following way. In the resting state, the cross-bridges, which are projecting parts of the myosin filaments, do not attach to the actin filaments, which are therefore free to

slide past them easily. This accounts for the high extensibility and relative plasticity of resting muscle. When the muscle is active, the cross-bridges can attach to specific sites on the actin filaments for a brief period of time, during which a relative force and, if the muscle is allowed to shorten, a relative movement are generated between the 2 types of filament, in some way at present unknown. The bridge then detaches and is free to form another attachment further along the actin filament if movement has taken place. Each bridge will perform a number of such cycles while the muscle is active (about 5 during a single twitch), the energy required for the process being liberated by the splitting of the substrate (probably ATP) by the ATPase of the myosin. When activity is over, the bridges cease to attach to the actin, enzyme activity comes to an end, and the muscle returns to the resting, relaxed state. When the muscle passes into rigor (a condition characterized by the absence of ATP), the cross-bridges become permanently attached to the actin filaments and the muscle is rigid and inextensible, for the filaments are not able to slide past each other.

#### Important Features of the Model

Reviews of the large amount of biochemical and physiologic data available concerning striated muscle and the structural model that has been described have already been published in extenso.<sup>8, 9, 10</sup> Here we will mention briefly only 3 particular points that seem worth while to emphasize.

1. The system can develop a range of different tensions depending upon the number of cross-bridges that are active simultaneously. For a given load, the system shortens with a particular velocity of shortening (rather than, say, a particular acceleration), such that the number of bridges actively developing tension is just sufficient to bear the load. The rate-limiting factor in the system is the rate at which unattached bridges can become attached again and develop tension; as a given active site on the

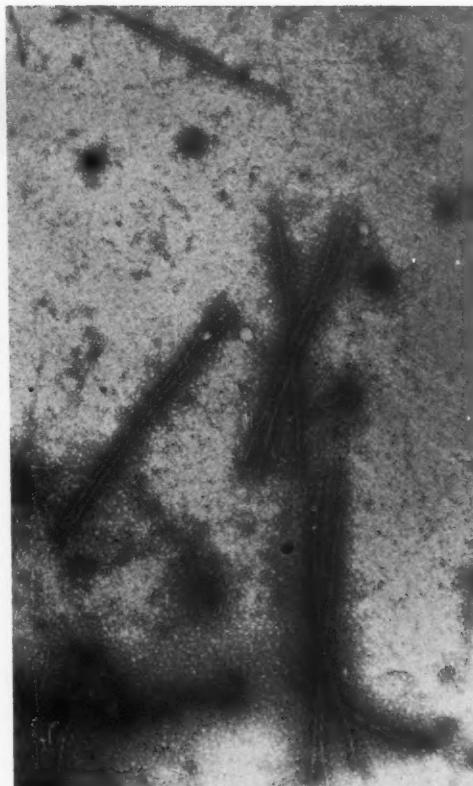


Figure 3

*Isolated thick filaments, 1.5 $\mu$  in length, prepared by blending glycerinated muscle in the presence of a relaxing agent (EDTA + ATP). Specimen prepared for electron microscopy by negative staining technic.  $\times 30,000$ .*

actin moves past the bridge, there is a certain length of time available for this process to take place: the faster the movement, the less the chance of attachment to a particular site, and the lower the tension at any given moment of time. Thus, when shortening under a particular load, the system will settle down to an equilibrium velocity at which the rate of formation of new links is just equal to the rate of opening of formed links that have already exerted their pull on the actin filament.

2. Energy release by the enzyme site is activated by the attachment of the cross-bridges to the actin filament. Thus the

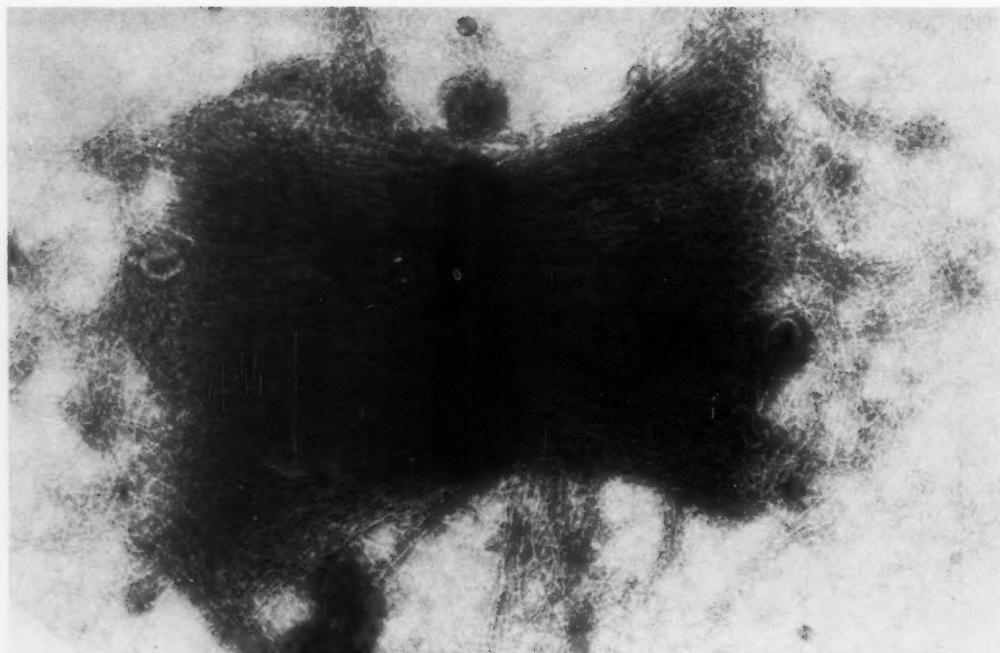


Figure 4

*Isolated I-segment (array of thin filaments) prepared as in figure 3.  $\times 60,000$ .*

number of fully active sites is controlled by the tension in the muscle, and the rate at which those sites repeat their cycles of activity is controlled by the velocity of shortening. The system therefore has the possibility of behaving economically, i.e., releasing more energy for a given distance of shortening when it is shortening against a larger load. This is a very important feature of the behavior of real muscles. One can see in a general way how such a system might give rise to the type of behavior characterized by the Hill equation:

$$(P + a)V = (P_0 - P)b$$

where  $P$  = actual load;  $P_0$  = isometric tension;  $V$  = velocity of shortening;  $a$  = constant (heat of shortening); and  $b$  = constant. The left side of this equation gives us the total rate of energy release required to do external work and produce shortening heat (assuming that maintenance heat can be accounted for separately). The right side contains the term  $(P_0 - P)$ , which is pro-

portional to the number of *unattached* bridges. If the attachment of such bridges is the rate-limiting step, as we have assumed, then the model could quite naturally give rise to this equation. A more elegant and complete account of the mechanical and thermal properties of striated muscle in terms of a particular version of the sliding filament model has been given by A. F. Huxley.<sup>9</sup>

3. It is not necessary to postulate that each cross-bridge generates a pull over a distance comparable to that separating successive cross-bridges between a given pair of thick and thin filaments, or between successive active sites on the actin filaments. All that is necessary is that the actin filament shall be drawn along such a distance *between 2* successive operations of a given cross-bridge, and this movement can equally well be achieved by small movements—say of 5 Å—produced successively at 10 other cross-bridges. As any given actin filament has

about 54 bridges directed toward it in each half of the A-band, this can be achieved quite easily, still leaving the possibility for up to 5 bridges to be acting in parallel at any given moment, to permit the variation in tension and rate of energy release with velocity of shortening that we have already described.

#### Special Features of Cardiac Muscle

Although the essential features of the contractile structure and its behavior appear to be the same in cardiac and skeletal muscles, there are a few points at which differences occur that may be significant. Probably the most obvious one is the presence of very large numbers of mitochondria in cardiac muscles, as compared to skeletal muscle, no doubt associated with the ability of the heart to function continuously over very long periods of time without intervals for recovery.

The second feature is the comparatively small diameter of heart-muscle fibers (as small as a few microns) compared with those of skeletal muscle, which are most commonly 50 to 100 microns in diameter. The latter are usually provided with quite an abundant reticulum, i.e., a system of internal membranes in each fiber, and these are believed to be concerned in relaying the signal for contraction into the interior. Such a reticulum is either very sparse or seemingly absent in many of the heart-muscle preparations that have been examined, a point of difference that, in view of the apparently different membrane properties of cardiac muscle, deserves further study.

Another rather puzzling feature of cardiac muscle is the relatively low tension per unit area which it will develop, only about one-tenth that of skeletal muscle according to 2 recent studies.<sup>11, 12</sup> Some of the difference may be accounted for by the greater fraction of the cross-sectional area occupied by mitochondria in the cardiac muscle—perhaps a factor of 2 difference might occur for this reason—but a large factor still remains, and there is no obvious reason from the

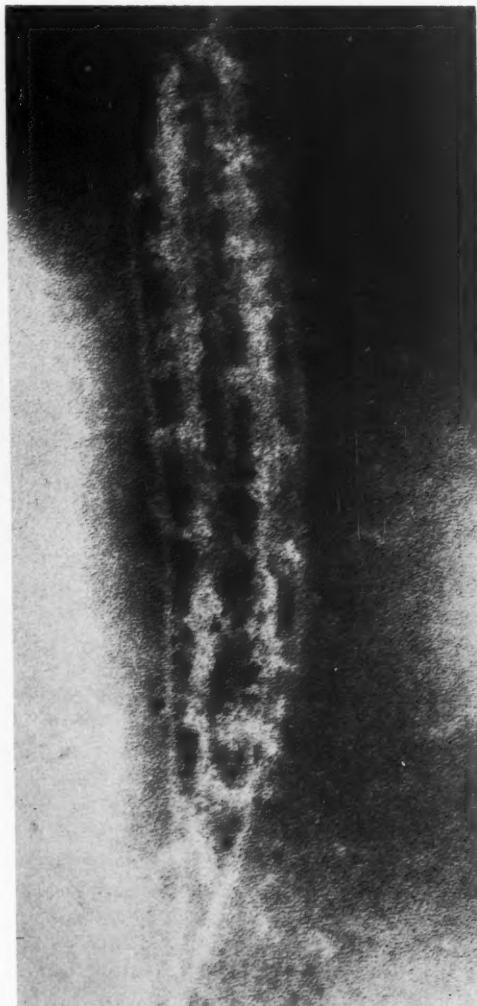


Figure 5

*Isolated group of thin and thick filaments, prepared as in figure 3, negatively stained.  $\times 300,000$ .*

visible structure to account for it. It may, of course, result from different enzymatic properties of cardiac actomyosin. This is a difficult hypothesis to investigate, as the enzymatic properties of all actomyosins seem to be rather low in comparison with what one would anticipate from the maximum energy output of the muscles from which they were obtained.

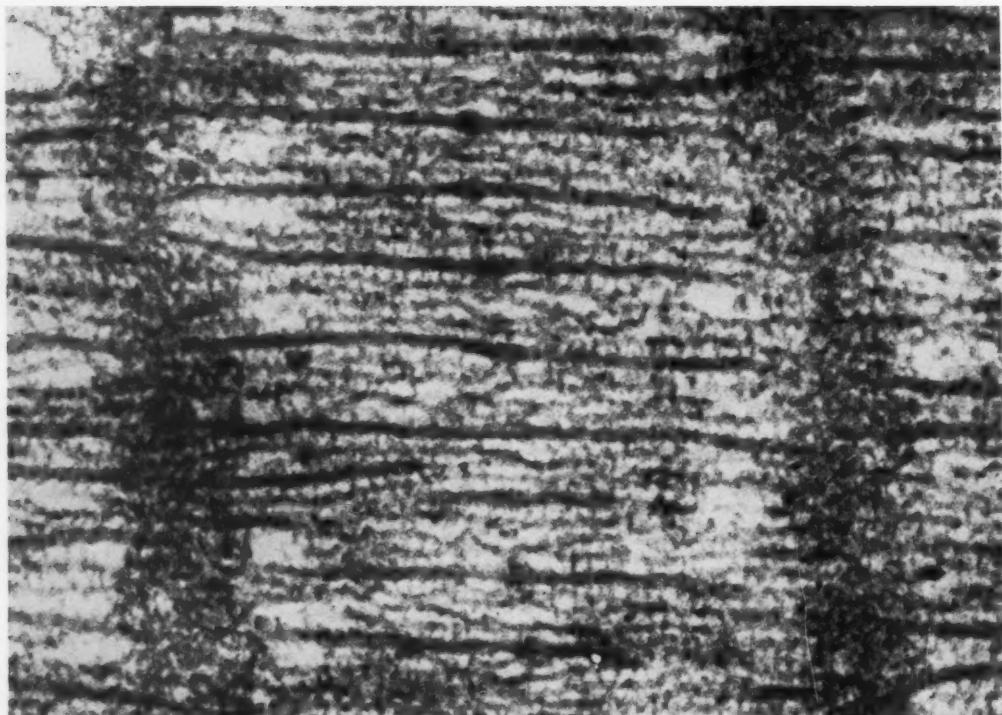


Figure 6

*Heavily contracted muscle, showing double overlap of thin filaments.  $\times 150,000$ .*

A fourth feature of cardiac muscle that distinguishes it from skeletal muscle is the nature of the active isometric length-tension curve, i.e., that showing the increase in tension over resting tension when the muscle is active. In skeletal muscle, this curve exhibits a maximum around resting length (which lies very close to the greatest length at which the muscle develops zero resting tension). As the length of the muscle is increased beyond resting length, the active tension decreases, an effect that has been explained by Huxley and Niedergerke<sup>6</sup> as resulting from the decreased length of the region in which actin and myosin filaments overlap and can form cross-links with each other. The factors that cause the tension to decrease below resting length are unknown. In cardiac muscle, however, the active tension *increases* as the length of the muscle is increased beyond resting length as defined

above, and reaches a peak only after a stretch of about 30 per cent.<sup>12</sup> This effect might be explained if the sarcomeres of cardiac muscle at resting length resembled, either in the extent of overlap or in the factors that produce the decrease in tension at length below rest length, those of skeletal muscle that had shortened by about 25 per cent.

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## The Sarcoplasmic Reticulum of Skeletal and Cardiac Muscle

By DON W. FAWCETT, M.D.

This paper traces the development of our present concept of the structural organization of the sarcoplasmic reticulum in striated muscle and reviews the physiologic evidence for its participation in intracellular impulse conduction. Comparative observations are presented showing that this system of membrane-limited tubules is particularly well developed in exceptionally fast-acting skeletal muscles. These findings are interpreted as evidence supporting the hypothesis that the reticulum is involved in the coupling of excitation to contraction, but it is considered likely that it also has other important functions in muscle metabolism. The sarcoplasmic reticulum of cardiac muscle is found to be much less extensive and less precisely arranged in relation to the cross-banded pattern of the myofibrils, than it is in skeletal muscle. It is believed, nevertheless, that it may prove to have a significant role in the physiology of the myocardium.

**A**MONG THE MOST SIGNIFICANT recent morphologic contributions to our understanding of muscle have been the demonstration by Huxley and Hanson<sup>1</sup> that the actin and myosin of the myofibrils form two distinct sets of interdigitating filaments, and the description by Bennett<sup>2</sup> and Porter and Palade<sup>3</sup> of the *sarcoplasmic reticulum*, a submicroscopic plexiform system of membrane-bounded tubules that occupies the interfibrillar spaces throughout the muscle fiber. The first of these discoveries has formed the basis for a new, and now widely accepted, sliding-filament theory of muscle contraction, and the second has defined a new organelle in the sarcoplasm that may play an important role in the coupling of excitation to contraction.

We propose to review the evidence for the current belief that the sarcoplasmic reticulum may be involved in intracellular impulse conduction and then to present some comparative observations on the organization of this system of membranes in certain examples of skeletal and cardiac muscle that have unusual physiologic properties.

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### Historical Considerations

Delicate intracellular networks surrounding the myofibrils were observed over half a century ago by Thin,<sup>4</sup> Retzius,<sup>5</sup> Veratti,<sup>6</sup> and a few other able cytologists in preparations of muscle stained by special metal-impregnation methods. However, the membranous nature of this system, its continuity throughout the sarcoplasm, and its exact relationship to the contractile elements could not be fully appreciated with the light microscope. The reticulum therefore aroused the interest of very few morphologists and was quite unknown to physiologists until it was rediscovered a few years ago by Bennett and Porter,<sup>7</sup> Andersson,<sup>8</sup> and Porter and Palade<sup>3</sup> in electron micrographs of skeletal muscle.

In these studies, electron micrographs of thin sections passing tangential to the myofibrils often revealed a plexus of smooth-surfaced tubules closely applied to their surface. From the examination of large numbers of micrographs of *Ambystoma* muscle, Porter and Palade arrived at an interpretation of the distribution of the sarcotubules that is presented diagrammatically in figure 1A. The tubules overlying the A band of each sarcomere are predominantly longitudinal in their orientation but communicate laterally with one another in the region of the H-band. At the ends of each sarcomere, the longitudi-

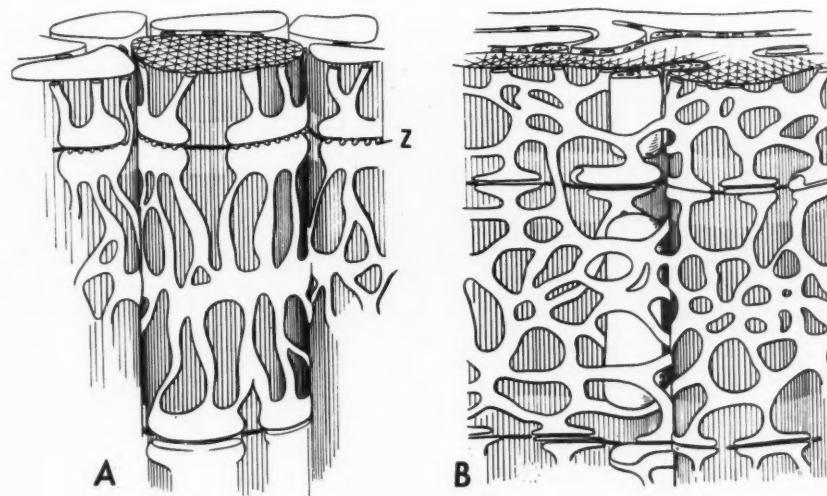


Figure 1

A. Diagrammatic interpretation of the organization of the sarcoplasmic reticulum in skeletal muscle of the salamander *Ambystoma punctatum*. Each myofibril is surrounded by a plexiform system of tubules. The tubules overlying the A-band are predominantly longitudinal in their orientation, but communicate freely in the region of the H-band. At the ends of each sarcomere the longitudinal tubules of the reticulum are confluent with dilated transverse channels called terminal cisternae. The complex oriented transversely at the Z-band, consisting of 2 terminal cisternae and an intermediate row of small vesicles or short tubules, is referred to as a triad. B. Diagram of the sarcoplasmic reticulum of rat cardiac muscle. The loose network of sarcotubes shows less regional differentiation in relation to the cross-banded pattern of the myofibrils. The terminal cisternae are small and typical triads are uncommon. (From Bennett, H. S.: In *Muscle*, vol. 1. New York, Academic Press Inc. Redrawn from Porter and Palade.<sup>3</sup>)

nal meshes of the reticulum are confluent with dilated, transverse channels called "terminal cisternae." The terminal cisternae of successive segmental units of the reticulum are situated on either side of the Z-band, separated by a row of small vesicles. The complex consisting of two terminal cisternae and the intermediate row of vesicles is referred to as a "triad" of the reticulum. In *Ambystoma* muscle these are oriented transversely or circumferentially with respect to each myofibril and are located on either side of the Z-line.

This system of sarcoplasmic tubules was interpreted by Porter and Palade as a special form of the *endoplasmic reticulum*, an organelle that they had described earlier in a wide variety of other cell types. In muscle, however, the reticulum lacked the ribonucleoprotein particles or ribosomes commonly associated with it in glandular cells and it was distributed in a very precise relation to the cross-banded structure of the myofibrils. This organization suggested to several investigators<sup>2, 3, 8</sup> that the reticulum might have a special role in muscular contraction, possibly providing pathways for preferential diffusion of metabolites or intracellular spread of excitation.

Physiologic evidence tending to support this latter speculation was soon provided by the ingenious experiments of Andrew Huxley and Taylor.<sup>9</sup> These investigators were concerned with the intracellular mechanisms whereby contraction of myofibrils deep in the interior of the muscle fiber is coupled to excitation of the surface membrane. In 1948, A. V. Hill<sup>10</sup> had concluded, from consideration of the rates and distances involved, that

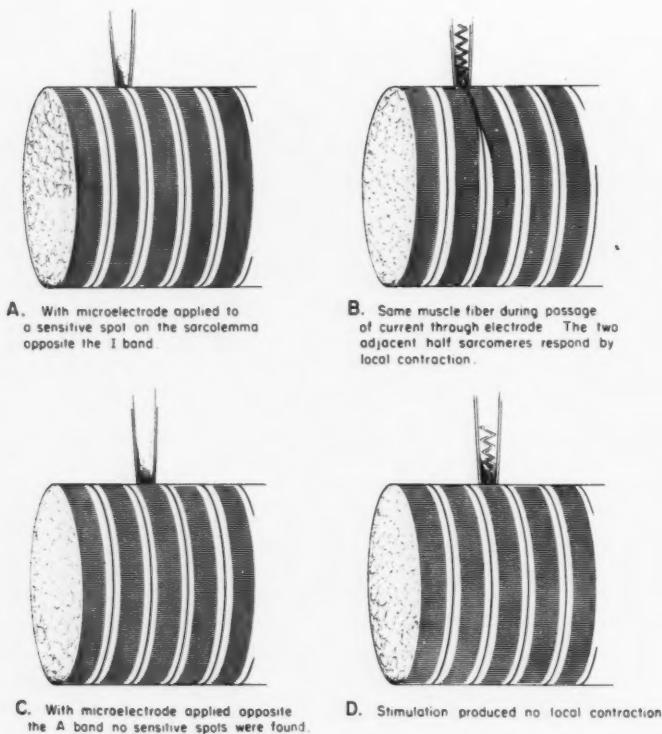


Figure 2

Diagrammatic representation of the experiments that suggested that some structural component located at the I-band of frog skeletal muscle was responsible for the inward spread of excitation. Stimulation with the microelectrode over the I-band often resulted in contraction of the adjacent half sarcomeres, whereas no contraction resulted from stimulation at the A-band. (Drawing based on illustrations in the paper by Huxley and Taylor.<sup>9</sup>)

the latency of response in skeletal muscle is much too short to be accounted for by the inward diffusion of a hypothetical activating substance from the sarcolemma to the contractile elements. Approaching this problem with new methods, Huxley and Taylor applied a microelectrode to different points on the sarcolemma of single frog muscle fibers under direct observation with an interference microscope (fig. 2). When the tip of the micropipette was over the I-band (fig. 2A), passage of current was often followed by contraction of the adjacent half-sarcomeres (fig. 2B), but no response was obtained when the stimulus was applied over the A-band (figs. 2, C and D). These results suggested that some structural component located in the I-band was responsible for the inward spread of excitation. The possibility that it was the Z-band itself was considered, but this had to be abandoned when similar experiments on

lizard muscle showed that the sensitive spots on the sarcolemma in this species were not at the level of the Z-band but over the A-band, near the A-I junction. Electron microscopic studies on the muscles of these two species revealed that the triads of the sarcoplasmic reticulum are situated at the Z-band in frog muscle but at the A-I junction in lizard muscle. The close correspondence between the position of the triads in the reticulum and the level in the sarcomere of spots sensitive to direct stimulation with a microelectrode strongly suggested that the impulse might be conducted inward by the membranes of the sarcoplasmic reticulum. This, then, is the historical background from which our own interest in the sarcoplasmic reticulum developed.

#### The Sarcoplasmic Reticulum of Fast-Acting Skeletal Muscles

If the sarcoplasmic reticulum is involved in the coupling of excitation to contraction,

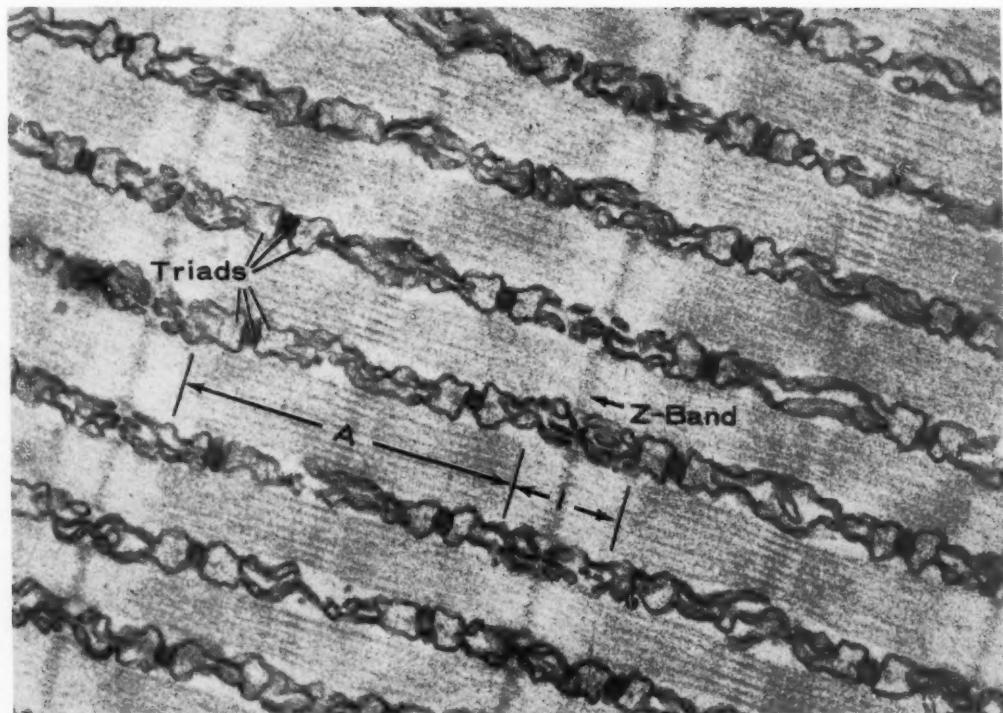


Figure 3

Longitudinal section of the sound-producing muscle in the swimbladder of the toadfish, *Opsanus tau*. In this unusually fast-acting muscle, the sarcoplasmic reticulum is exceptionally well-developed. There are 2 triads to each sarcomere length located over the A-band near the A-I junctions. Longitudinal tubules extend in either direction from each triad toward the H- and toward the Z-band respectively. The longitudinal elements of the reticulum are continuous over the H-band but often appear to be interrupted at the Z-band (see at arrows). (Electron micrograph by Dr. J. P. Revel.)

one might expect to find differences in its organization or its degree of development in muscles having different speeds of contraction. With this in mind, Dr. Jean Paul Revel and I have studied the reticulum of some particularly fast-acting muscles.

The first of these is a muscle that forms an equatorial band around the swim bladder of the common toadfish, *Opsanus tau*. Rapid contractions of this muscle set up vibrations in the taut gas-filled bladder that produce the audible sounds made by these fish when they are courting or when otherwise disturbed. This muscle is said to attain its peak contraction in only 5 to 8 msec.<sup>11</sup> and requires some

300 stimuli per second to tetanize.<sup>12</sup> Since the fine structure of this muscle has been described in detail in a separate publication,<sup>13</sup> only a brief account of its salient features will be presented here. The fibers are of large diameter. Their myofibrils are flat ribbon-like structures arranged radially around a central core of sarcoplasm to form a thick-walled contractile cylinder. Mitochondria are seldom found between the myofibrils. Instead, they are located either in the core of the contractile cylinder or in the superficial layer of sarcoplasm around its periphery. The narrow clefts between the broad faces of the myofibrils are occupied by a highly developed

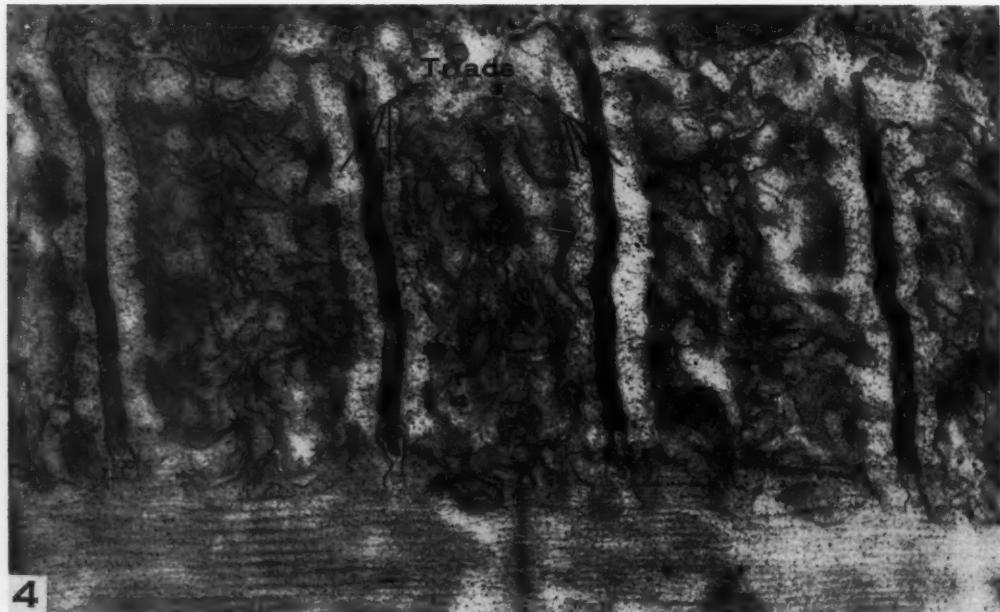


Figure 4

Electron micrograph of a thin section passing through toadfish muscle, parallel to the broad face of a myofibril. The triads are seen running across the myofilaments parallel to each other and to the Z-band. Crowding and superimposition tend to obscure the plexiform nature of the reticulum between the successive triads. The middle element of the triad, which appears here as a row of small vesicles, is actually a continuous slender tubule.

sarcoplasmic reticulum. The ribbon-like myofibrils present their narrowest dimension in a longitudinal section through the wall of the contractile cylinder (fig. 3). There is a long A-band and a rather short I-band, and these are precisely aligned across the entire width of the fiber. The reticulum in the interfibrillar clefts is extremely regular in its organization and shows two triads in each sarcomere length. These are invariably located near the A-I junctures where the two interdigitating sets of filaments described by Hugh Huxley<sup>14</sup> are presumed to slide with respect to one another during muscular contraction. The triads run transversely across the broad face of the myofibrils and radially with respect to the contractile cylinder as a whole. Thus in most longitudinal sections of the muscle fibers the triads are seen in cross section (fig. 3). Each

consists of a slender intermediate tube, approximately 30 m $\mu$  in diameter, flanked by two larger cisternae about 110 m $\mu$  across. The longitudinal tubules connecting successive triads run parallel to the myofibrils and anastomose freely to form reticula in two or more layers that are closely applied to the surfaces of the adjacent contractile elements.

Sections passing through the interfibrillar clefts, in which the triads are cut longitudinally as they traverse the broad face of the underlying myofibril, provide a more extensive view of the reticulum (fig. 4). In this view, the intermediate element often appears as a row of vesicles, but in the best preserved specimens it seems to be a continuous tubule. In the intervals between triads, the plexiform nature of the longitudinal elements of the reticulum is often obscured by superimposition



Figure 5

*An electron micrograph of a section passing tangential to a myofibril in the cricothyroid muscle of the bat, Myotis lucifugus. In this fast-acting mammalian muscle too, the sarcoplasmic reticulum is far more elaborately developed than in slower muscles. The triads are located at the A-I junctions and the longitudinal sarcotubules seem to be continuous across the Z-band as well as across the H-band. (Micrograph by Dr. J. P. Revel.)*

of more than one layer of sarcotubes, so that it is difficult to ascertain whether the reticulum is continuous from sarcomere to sarcomere across the Z-band or only between triads within the same sarcomere. At the outer margin of the contractile cylinder the cisternal elements of the triad narrow abruptly and follow a sinuous course among the mitochondria in the peripheral layer of sarcoplasm. Some of them can be followed to the sarcolemma. It is assumed that such points of contact of the reticulum with the surface membrane may correspond to the sensitive points found in frog and lizard muscle with the searching microelectrode in Huxley and Taylor's experiments.

Seeking a fast-acting mammalian muscle for study, Dr. Revel, in our laboratory, has examined the cricothyroid muscle of the bat.<sup>15</sup> Physiologic measurements of the time course

of contraction in this muscle have not been made, but one can infer from its normal function that it is very fast. In its sonic navigation, the bat uses pulses of supersonic sound of the order of 5 to 10 msec. in duration and within this brief period it is capable of modulating the frequency over a considerable range. To accomplish this, the cricothyroid must be able to change its state of contraction very rapidly. When examined in electron micrographs of low magnification, the appearance of this muscle does not differ greatly from other mammalian muscles. The mitochondria are numerous and arranged in rows between myofibrils of the usual rounded or polygonal cross-sectional shape. At higher magnification, it is evident that the sarcoplasmic reticulum is exceptionally well developed (fig. 5). The transverse triads near the A-I junctions appear to encircle the myo-

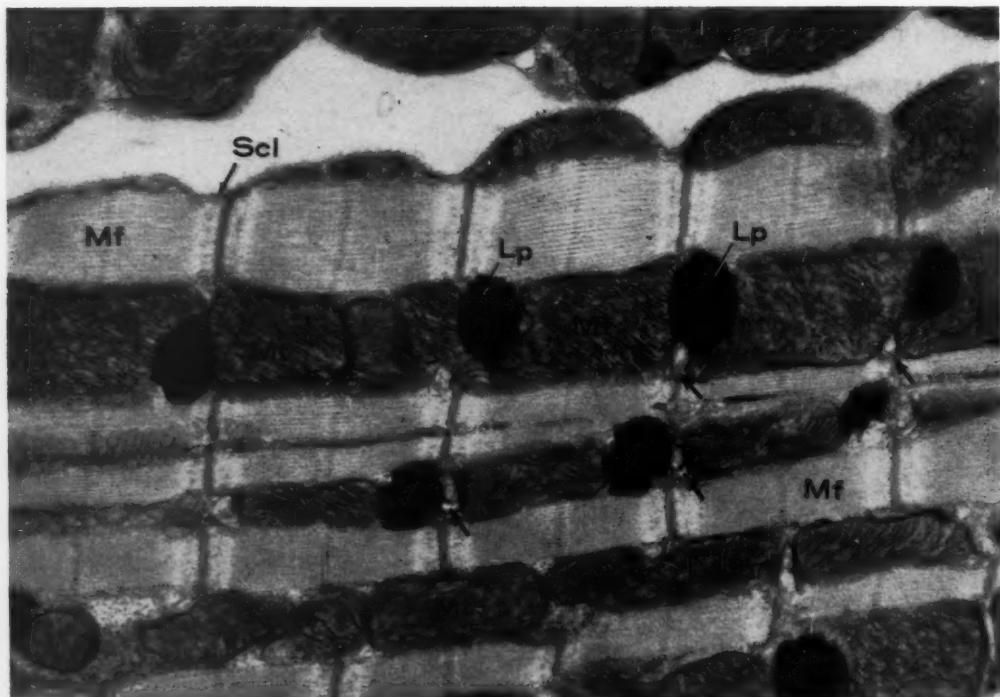


Figure 6

*Longitudinal section of a peripheral portion of a cardiac muscle fiber from the bat heart. Many large mitochondria (MT) are located immediately beneath the sarcolemma (Scl) and between the myofibrils (MB). Numerous lipid droplets (Lp) among the mitochondria are evidently used as an energy source. At several places indicated by arrows, transverse elements of the sarcoplasmic reticulum corresponding to the triads of skeletal muscle are seen between the mitochondria at the level of the Z-band.*

fibril completely and are, no doubt, continuous with the triads of adjacent myofibrils. Their cisternae are more slender than those of the fish muscle described earlier, and the intermediate element more commonly appears to be a narrow continuous tube. The longitudinal sarcotubules of the reticulum are also of smaller and more uniform caliber and they seem clearly to be continuous from sarcomere to sarcomere across the Z-band. Occasionally there is a partial reduplication of a "triad" resulting in a "pentad" consisting of 3 cisternae and 2 slender intermediate tubules.

The finding of an unusually extensive and highly ordered sarcoplasmic reticulum in these 2 exceptionally fast-acting skeletal muscles is consistent with the hypothesis<sup>16</sup> that this

system of membrane-bounded channels is involved in intracellular conduction of the impulse that activates the myofibrils.

#### Sarcoplasmic Reticulum of Cardiac Muscle

Several considerations would lead one to expect that the sarcoplasmic reticulum might be less well developed in cardiac than in skeletal muscle. Some of these are: the smaller fiber diameter; the central position of the nucleus, which brings the contractile elements nearer to the surface; the myogenic nature of the contraction; the presence, at frequent intervals along the cardiac muscle fibers, of specialized cell-to-cell junctions (intercalated discs) that may offer pathways of inward conduction from the sarcolemma, not present

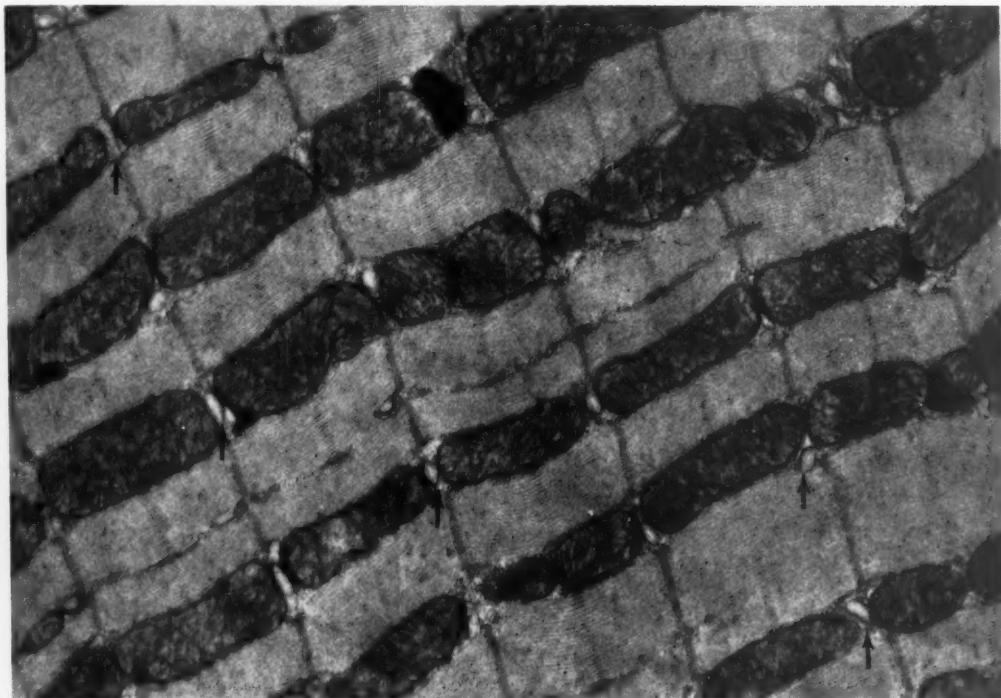


Figure 7

Another longitudinal section of the interior of a cardiac muscle fiber from the bat heart showing contracted myofibrils alternating with rows of mitochondria, each about the length of one shortened sarcomere. Again the arrows point out transverse elements of the reticulum located at the Z-band instead of at the A-I junction, which is the usual location of the triads in mammalian skeletal muscle.

in the syncytial fibers of skeletal muscle; and finally the slower rate of contraction of heart muscle. Nevertheless, there is sufficient correlation between the rate of heart beat in various animal species and the degree of development of the sarcoplasmic reticulum to suggest that this system has a significant function in cardiac as well as in skeletal muscle. In a previous study of the fine structure of the turtle atrium,<sup>17</sup> the reticulum was found to be rudimentary. Evidently it is not essential in the slow-beating heart of this cold-blooded species. In the rat, which has a rather rapid heart rate, Porter and Palade<sup>3</sup> found a loose network of sarcotubes with relatively little differentiation in relation to the cross-banded pattern of the myofibrils (fig.

1B). Although small terminal cisternae were identifiable on either side of the Z-band, these did not form typical triads nor did they extend laterally for any considerable distance.

We have recently studied the myocardium of the bat, *Myotis lucifugus*. These small mammals normally have a heart rate of the order of 500 to 600 per minute but, under some physiologic conditions, it may reach as high as 1,000 per minute. Electron micrographs of longitudinal sections reveal an extraordinary number of large mitochondria of complex internal structure located in the clefts between myofibrils, at the poles of the centrally placed nucleus, and immediately beneath the sarcolemma.

The mitochondria are often about the length

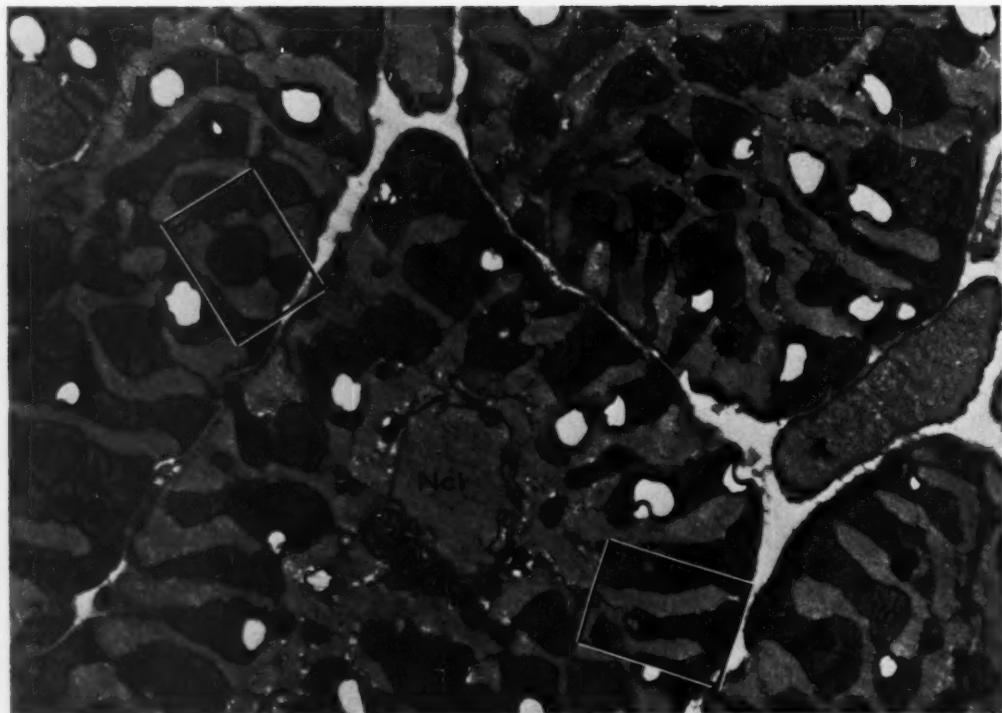


Figure 8

*A low power electron micrograph of portions of 4 cardiac muscle fibers of the bat in transverse section. Observe the centrally placed nucleus (Ncl) and the fact that the large dense mitochondria occupy fully half the cross-sectional area of the fiber. The clear areas that appear to be holes in the section are in fact lipid droplets whose content has been largely extracted during specimen preparation. The area enclosed in rectangle A is shown at higher magnification in figure 9 and that in rectangle B constitutes figure 10.*

of a sarcomere. The periphery of a partially contracted fiber frequently shows a characteristic scalloped or corrugated appearance owing to the fact that the sarcolemma is closely adherent to each Z-band of the outermost myofibrils but is separated from the myofibrils elsewhere by mitochondria. The mitochondria immediately beneath the sarcolemma would thus seem to be confined within relatively stable compartments, bounded by the lines of adhesion of the sarcolemma to the Z-band of successive sarcomeres (fig. 6). The structural basis for this close binding of the surface membrane to the Z-band is not clear from the micrographs. Numerous lipid droplets are interspersed among the mitochondria that are

more deeply situated in the fibers (fig. 6) and are evidently an important energy source in the rapidly beating hearts of this and other small mammals. Since bats hibernate, it would be of interest to know whether there are seasonal variations in the abundance of the myocardial lipid, but, thus far, our studies do not extend over a large enough span of time to throw any light on this subject.

Also located between the ends of the mitochondria at the level of the Z-band are transversely oriented tubular elements of the sarcoplasmic reticulum, indicated by arrows on figures 6 and 7. These evidently correspond to the triads of the reticulum of skeletal muscle but tend to be single or at most double and

are placed at the Z-band instead of in the A-band near the A-I junction. One tends to underestimate the extent of the sarcoplasmic reticulum in cardiac muscle because of the peculiar geometry of its myofibrils. They are not discrete fibrils uniformly round or polygonal in cross section, as in skeletal muscle, but instead exhibit a greater degree of confluence and branching so that, in transverse sections, the size of the myofibrils is variable and their shape highly irregular. In consequence of the inconsistency of their tridimensional form and the prevalence of curving surfaces, one rarely encounters such extended surface views of the sarcoplasmic reticulum in longitudinal sections as one sees overlying the more regular faces of the myofibrils in skeletal muscle. It is necessary therefore to rely mainly on cross sections in attempting to construct a mental image of the 3-dimensional organization of the reticulum.

In transverse sections of bat heart muscle viewed at low magnification (fig. 8), one is struck by the irregular shape of the myofibrils around the centrally placed nucleus and by the great number of large mitochondria that take up nearly half of the cross-sectional area of the fiber and occupy nearly all of the interfibrillar sarcoplasm. In micrographs of higher magnification (figs. 9 and 10) the mitochondria are found to conform very closely to the irregular contours of the surrounding myofibrils; however, in the narrow interstices between the two, there are numerous circular profiles, 400 to 500 Å in diameter (see at arrows), which are cross sections of the longitudinally oriented tubules of the sarcoplasmic reticulum. Owing to the paucity of interfibrillar sarcoplasm, these profiles are easily overlooked in low-power micrographs but, from the large numbers visible in micrographs of higher magnification, it is clear that the sarcoplasmic reticulum is quite well developed in cardiac muscle of this animal species.

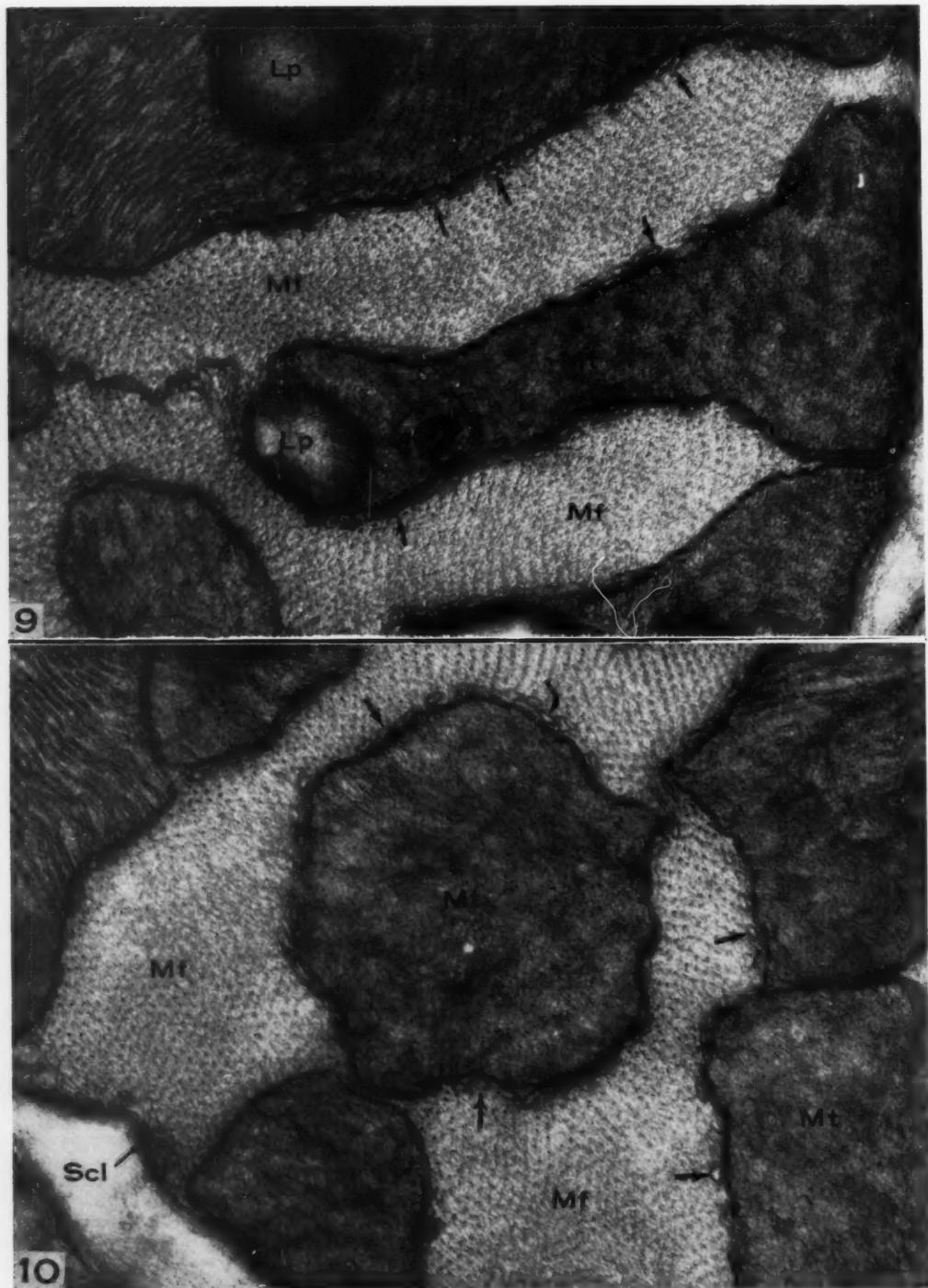
To what extent is this tubular system developed in the human heart? Although there have been several brief reports on the fine

structure of the human myocardium,<sup>18</sup> none has devoted particular attention to the sarcoplasmic reticulum. Our own studies on man are as yet too fragmentary to permit us to do more than to affirm its presence and to record some preliminary impressions on the degree of its development as compared to other animal species.

In electron micrographs of human atrial muscle, the myofibrils show the same orderly arrangement of two interdigitating sets of filaments that has been described in other striated muscles (fig. 11). The myofibrils vary considerably in size and in cross-sectional shape but are, on the whole, less pleomorphic than those described here for the bat. The mitochondria, which have a dense matrix and a complex internal membrane structure, are numerous and are distributed singly or in sizeable clusters among the myofibrils. The interfibrillar sarcoplasm is more abundant than in the bat myocardium. The mitochondria, being less crowded, show less tendency to adopt unusual shapes conforming to the spaces between the myofibrils. Among the mitochondria and in the clefts between adjacent myofibrils are tubular elements of a sparse sarcoplasmic reticulum (see arrows, fig. 11). Definite triads have not been identified at the level of the Z-bands in our material, nor have any clear connections been demonstrated between the loose meshes of the reticulum and the sarcolemma. Although it is probably basically similar in its distribution and organization, the reticulum in the human myocardium is evidently far less elaborately developed than is that of smaller mammals with a more rapid heart beat.

#### Comment

The history of the discovery of the sarcoplasmic reticulum of striated muscle has been traced and the physiologic evidence for its participation in intracellular impulse conduction has been reviewed. Our own comparative observations indicate that this system of membrane-limited tubules is particularly well developed in certain exceptionally fast-acting skeletal muscles. These findings



Figures 9 and 10 (See legend on opposite page)

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Figure 11

An electron micrograph of a small area at the periphery of a human cardiac muscle fiber. The sarcolemma (ScL) is at the top of the figure and shows the usual coating of basement-membrane material. The mitochondria (Mt) are fairly large and rich in internal structure. The myofibrils (Mf) show the usual precise hexagonal pattern of myofilaments. The sarcoplasmic reticulum is less well developed than in the hearts of small mammals with rapid heart beats, but several profiles of sarcotubules in cross section can be seen between the myofibrils in this figure (see at arrows).

are offered as further indirect evidence for the hypothesis that the reticulum is involved in the coupling of excitation to contraction. It is recognized, however, that this canalicular system may function in other ways besides the conduction of an impulse by its limiting membrane. It may prove to be important in

the synthetic activities of the muscle cell, or its lumen may provide a continuous pathway for distribution of energy-rich compounds or other essential metabolites to the myofibrils.

The sarcoplasmic reticulum has been shown to be less highly developed in cardiac than in skeletal muscle but it is so organized in

#### Figures 9 and 10

Higher magnification electron micrographs of two small areas of the cardiac muscle fiber shown in figure 8. The myofibrils (Mf) are highly irregular in shape and their surface closely conforms to the shape of the mitochondria (MT), which occupy nearly all of the intermyofibrillar sarcoplasm. Between the myofibrils and the mitochondria are numerous small profiles of membrane-bounded tubules in cross section (see at arrows). These are the longitudinal components of the sarcoplasmic reticulum.

relation to the cross-banded pattern of the myofibrils as to suggest that it may have a similar function in both. The reticulum is rudimentary in the slow-beating heart of the turtle but reaches a rather high degree of complexity in the very rapidly beating heart of the bat. The reticulum of the human myocardium has not been adequately studied but appears to be intermediate between these extremes. It is not possible now to state how important a role the sarcoplasmic reticulum plays in the physiology of the human heart, but new findings in research often turn out to have far more significance than at first seems likely. It may not be too fanciful to imagine that a few decades hence the cardiologist may be concerned with functional disturbances of this intracellular communication system just as he is concerned today with defects of conduction at a grosser tissue level.

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# The Structure of the Specialized Impulse-Conducting System of the Steer Heart

By JOHANNES A. G. RHODIN, M.D., PETER DEL MISSIER, M.D.,  
AND L. CORSAN REID, M.D.

The specialized impulse-originating and -conducting system of the steer heart has been analyzed with the light, phase contrast, and electron microscopes after careful dissection of its gross anatomic parts. The specific tissue is composed of cells with distinct cell boundaries. No syncytium exists. The cells of the sino-atrial (S-A) and atrioventricular (A-V) nodes closely resemble those of the common myocardium. They connect end to end via intercalated disks, most of which represent the starting point and termination of numerous myofibrils. The cells of the bundle of His and its distal branches are large and spindle-shaped and joined in a staggered fashion. They display a fair number of myofibrils. The cell contact is established by numerous desmosomes that only rarely connect with myofibrils. It is believed that the multiple desmosome type of connection, present in most parts of the specific tissue of the steer heart, indicates that this tissue has maintained its embryologic appearance, to a large extent. The role of the desmosomes in facilitating the propagation of the impulse throughout the specific tissue is discussed.

IT SEEMS APPROPRIATE to discuss the structure of the specialized conducting system of the heart at a symposium on the myocardium. Without this tissue, the stimulus for the contraction of the common myocardium would hardly ever be originated. Thus, it is to be expected that certain unique peculiarities will characterize the cells of the specialized conducting system. An account of their ultrastructure will be given here in the hope that this will explain, among other features of this tissue, its ability to transmit the impulse of contraction so much faster than the cells of the common myocardium.

Although the most fundamental mechanism of this system is the origination of an impulse, there has been a long-lasting controversy regarding its existence as a specific tissue in the heart.<sup>1, 2</sup> This is due principally to its great variation in style and structure throughout the animal kingdom, including mammals. At present this controversy is settled and the existence of a specific tissue in man,<sup>3, 4</sup> sheep,<sup>5, 6</sup> and steer<sup>7</sup> is structurally and func-

tionally beyond any doubt. In the reader's own mind, however, it may not be quite clear whether or not the specific tissue is muscular or nervous. Here, new technics such as phase-contrast and electron microscopy have been most helpful in demonstrating that the so-called conducting system is a true contractile muscular tissue.

## Material and Methods

The material used in this study has been exclusively the specific tissue of the steer heart. The hearts were obtained and dissected in the slaughter house within 15 minutes of the animal being killed. Osmium tetroxide was used as a fixative<sup>8, 9, 10</sup> and liquid plastic as an embedding medium<sup>11</sup> for specimens prepared for electron microscopy. Thick sections for phase-contrast microscopy and thin sections for electron microscopy were cut with the LKB Ultrotome.<sup>12</sup> The Siemens Elmiskop I electron microscope was employed.

## Results

### Anatomy of the Conducting System

The S-A node is regarded as the pacemaker, often called the node of Keith-Flack. In man, as well as in the steer, there is no direct continuity via specific tissue between the cells of the S-A node and the A-V node, of which the latter forms the beginning of the ventricular part of the conducting system. In the

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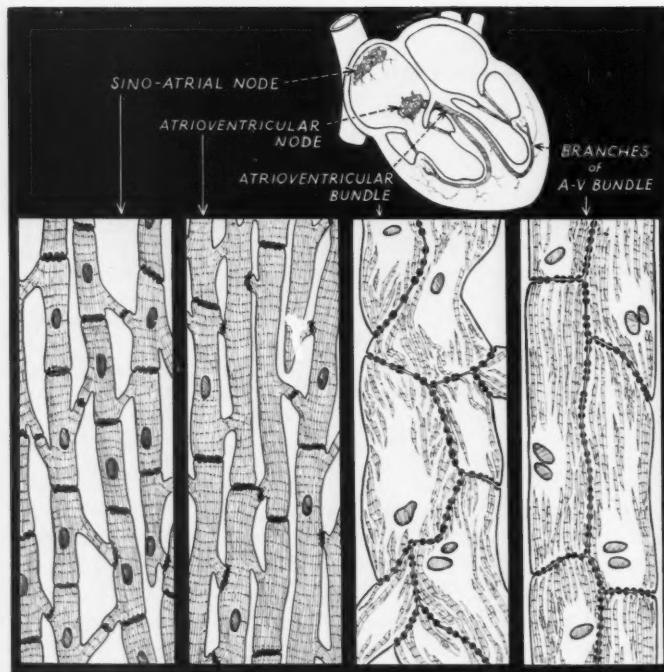


Figure 1

A schematic representation of the gross anatomic, histologic, and to some extent the ultrastructural organization of the conducting system of the steer heart. The four areas represent camera lucida drawings at a magnification of about 130 times. The size of the intercalated disks and the desmosomes is somewhat exaggerated in order to facilitate a comparison of their ultrastructure.

steer,<sup>7</sup> the A-V node or node of Tawara is quite large and diamond-shaped (fig. 1). The A-V node is followed directly by the A-V bundle also called the "common bundle" or the "bundle of His." This structure eventually splits up into a left and a right branch, each of which in turn gives rise to a network of fine ramifications that terminate in direct contact with the myocardial fibers.

#### Structure of the Common Bundle

##### Light Microscopy

The structure of the common bundle will be considered first, for, in the present material, the cells that compose this part of the conducting system are easily recognized because of their pronounced difference in shape and size from those of the common myocardium, justifying the term "specific tissue." This difference is easily seen even with the light microscope (fig. 2). Here, the size of the specific-tissue cells can be compared with that of the common myocardium. It is obvious that the cross-section diameter of the

specific cells is at least twice that of the cells of the common myocardium. The common bundle is rich in connective-tissue elements. No true inclosing of connective tissue can be identified, as in the sheep heart. The individual fibers of the common bundle are, however, sharply delimited from the surrounding connective tissue, as seen in cross section in the phase-contrast microscope (fig. 3). The interior of the fiber is divided by delicate lines that give rise to irregularly shaped areas. In a longitudinal section of a similar fiber (fig. 4), the lines and areas can be recognized. Nuclei are present in several areas or fields. In addition, there are longitudinally arranged fibrils that bear a well-defined cross striation. In other words, we are dealing here with a conducting fiber that is composed of a number of cells put together not only end to end, but also side to side in a staggered position because of their obvious spindle or multiangular shape. Because of the cross-striated appearance of the cellular fibrils, it is evident that the cells represent muscle cells.

*Electron Microscopy*

The cells of the conducting-system fibers are about 100 to 200 microns long in the common bundle. In the coarser fibers, 5 to 7 cells may be seen in a cross section (fig. 3) but finer fibers with only two cells also occur (fig. 5). The fiber is surrounded by a thin basement membrane with a varying number of reticular and collagenous fibrils. The basement membrane does not penetrate between the individual cells that build up the fiber. The boundaries of cells are difficult to see in the low magnification electron micrographs (figs. 5 and 6) but their course can easily be traced because of the relatively heavy accumulation of cross-striated myofibrils along their surfaces. Indirectly, the various cell territories can be distinguished also by the variation in density of the cytoplasm in different cells (fig. 6).

The cells of the common bundle have few cytoplasmic organelles as compared with the cells of the common myocardium. More than 50 per cent is occupied by what the early cytologist would have called "ground cytoplasm." In the electron micrograph, this part of the cytoplasm is characterized by a fine granularity, with the granules having an average diameter of about 200 Å (fig. 7). This is close to the diameter of the submicroscopic granules that are abundant in the exocrine cells of the pancreas, where they have been demonstrated to contain ribonucleoprotein.<sup>13</sup> However, histochemical techniques have shown that the light microscopically "clear" areas of the specific-tissue cells do contain glycogen.<sup>6</sup> The fine granularity is therefore believed to represent glycogen, similar to that which has been demonstrated in the myocardium of the turtle atrium.<sup>14</sup>

Another submicroscopic component is represented by small vesicles of varying size. The vesicles have a clear center and are bordered by a smooth membrane (fig. 7). They do not seem to be derived from the cell membrane, and their function is unknown. They may be abundant in one cell, and completely absent in another (fig. 6), which possibly indicates variation in function.



Figure 2

*Light micrograph of the common bundle (C) and the myocardium (Y). The cells of the common bundle are large and pale, whereas those of the myocardium are small and dense. (Formalin fixation; hematoxylin-eosin stain;  $\times 250$ .)*

Other cell components that can be seen with the aid of the light microscope are nuclei, mitochondria, myofibrils, and occasional lipid droplets. There is more than one nucleus to each cell. The nuclei usually occur in pairs and are located in the center of the cells of the common bundle. A heavily stained nucleolus, which has a loose structure, is always present (fig. 6).

The *mitochondria* are small and spherical. They display the common fine structural pattern of a thin triple-layered outer membrane and several triple-layered inner membranes (fig. 7). The matrix of each mitochondrion is quite loose in the present material. This may indicate less good preservation than desired and could depend on the relatively long lapse of time between the killing of the steer and the moment when



Figure 3

*Phase-contrast micrograph of a cross-sectioned fiber of the common bundle. The fiber is sharply delimited toward the surrounding connective tissue. The lines in the interior of the fiber represent cell borders. (Osmium tetroxide fixation; unstained plastic section, X 500.)*

these delicate and sensitive organelles were impregnated by the slowly penetrating osmium tetroxide. The mitochondria are usually found along the cell borders and also closely associated with the myofibrils.

The most prominent components of the cells of the common myocardium are the *myofibrils*. They vary greatly in length and width (figs. 6 and 7). Most are freely dispersed in the cytoplasm, but some begin and terminate at the cell membrane, here in close association with the so-called desmosomes. This arrangement is, however, much more pronounced in the distal branches of the conducting system. Only rarely have structures been identified that resemble the Purkinje filaments demonstrated by Muir in the sheep heart.<sup>6</sup>

Each myofibril shows the fine structure (fig. 7) that has been described in both the skeletal<sup>15, 16, 17</sup> and the heart muscle.<sup>14, 18</sup> Myofilaments are the main components with the traditional thickenings associated with the various bands (see Huxley in this symposium). The Z-bands are of particular interest in the myofibrils of the specific-tissue cells. In the cells of the common myocardium and the skeletal muscle, this structure is known to be composed of split myofilaments, embedded in an amorphous, electron-dense substance. In the present material, it has been found that every now and then the dense amorphous substance extends beyond the territory of the myofibril and establishes a direct continuation with a similar dense amorphous band that in some instances accompanies the cell borders at a distance (fig. 7). In order to understand this relationship fully it will be necessary to develop the concept of an intercellular relationship in the common bundle.

The *cell border* follows an irregular and wavy course (figs. 7 and 8). The plasma membrane of each cell is quite delicate and seems to be thinner than recorded for cells elsewhere in the body.<sup>19</sup> Two structures are associated with it: One is represented by the just-mentioned continuous electron-dense band, which roughly follows the course of the cell border, although some distance removed. The second structure is closely associated with the plasma membrane and is attached for short interrupted areas at its intracellular aspect. The plasma membrane of either specific-tissue cell is about 50 Å thick, and the intercellular space, characterized by less electron density, measures about the same. As the dense structures associated with the intracellular aspect of either plasma membrane are approached, the intercellular space widens to about 175 Å and becomes occupied by a dense substance in which, possibly, cross striations may be distinguished. At higher magnification, another membranous layer can be resolved, which is embedded in the cytoplasmic dense zone (fig. 9). This second layer is identical in size with the

plasma membrane and also parallel to it. The entire structural complex has been analyzed and described previously in the ventricles of frog, mouse, and guinea-pig hearts<sup>17, 20-22</sup> in the frog heart and papillary muscle of the dog heart,<sup>23</sup> and in the turtle atrium.<sup>14</sup> Sjöstrand and co-workers<sup>17, 20-22</sup> refer to this structure as the S-region, whereas the school of Fawcett<sup>14</sup> prefers the more commonly accepted term "desmosome." We should like to use the term "desmosome" for this structure in the common bundle of the steer heart because it closely resembles the desmosomes seen in a variety of epithelial cells, most advantageously in the epidermis.<sup>24-26</sup> If we now return to the *continuous dense band*, it becomes evident that this structure is a cytoplasmic condensation, similar in appearance to the desmosomes. It is obvious that, in the present material, the dense band is not applied to the plasma membrane but is parallel to it, and that it can be seen to connect desmosomes with each other. Every so often a connection is also established with the Z-band of a nearby myofibril. In concluding, it should be stressed that the cells of the specific tissue as analyzed in the common bundle of the steer heart are provided with desmosomes, the great number of which was not previously known. The functional interpretation of this arrangement will be discussed after we have considered the cellular contacts as they appear in the S-A and A-V nodes.

#### Structure of the Distal Branches of the Common Bundle

As the distal branches of the A-V bundle are approached, the cells become longer. They meet preferably end to end in addition to their side-to-side contact (fig. 10). The number of myofibrils increases considerably, and they are definitely longer and more parallel in arrangement when compared to the cells of the common bundle. The increased number of myofibrils gives less space for accumulation of glycogen. The nuclei also appear in pairs here, mostly located toward one end of the cell and close to the lateral cell border.

The fine structure of these cells is identical

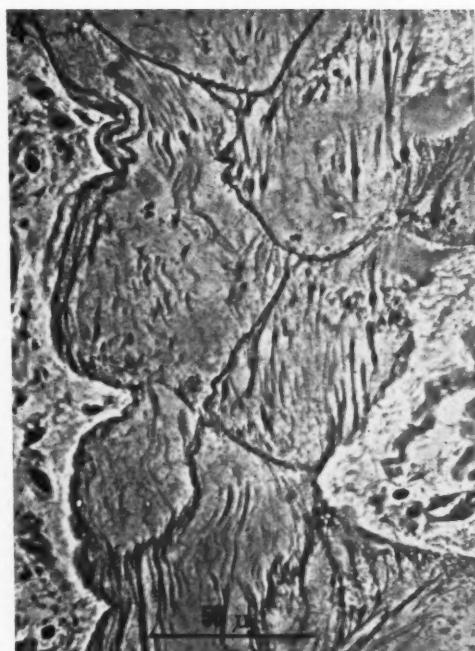


Figure 4

Phase-contrast micrograph of a longitudinally sectioned fiber of the common bundle. The spindle-shaped cells are arranged in a staggered fashion. A number of cross-striated myofibrils are present. (Osmium tetroxide fixation; unstained plastic section;  $\times 500$ .)

with that of the common bundle cells. Among other things, this implies that the multiple desmosome type of connection is maintained. The dense band with a course parallel to the plasma membrane is seen less often.

#### Structure of the S-A and A-V Nodes

##### Light Microscopy

In the steer heart, earlier investigators<sup>27</sup> have demonstrated by light microscopy that the cells of both nodes resemble those of the common myocardium. Grossly one can tell them apart because the nodal fibers are surrounded by an abundance of connective-tissue elements and by the comparatively loose arrangement of individual fibers (fig. 11). These circumstances have been used as criteria in the present study.

In a cross section of S-A fibers (fig. 12),

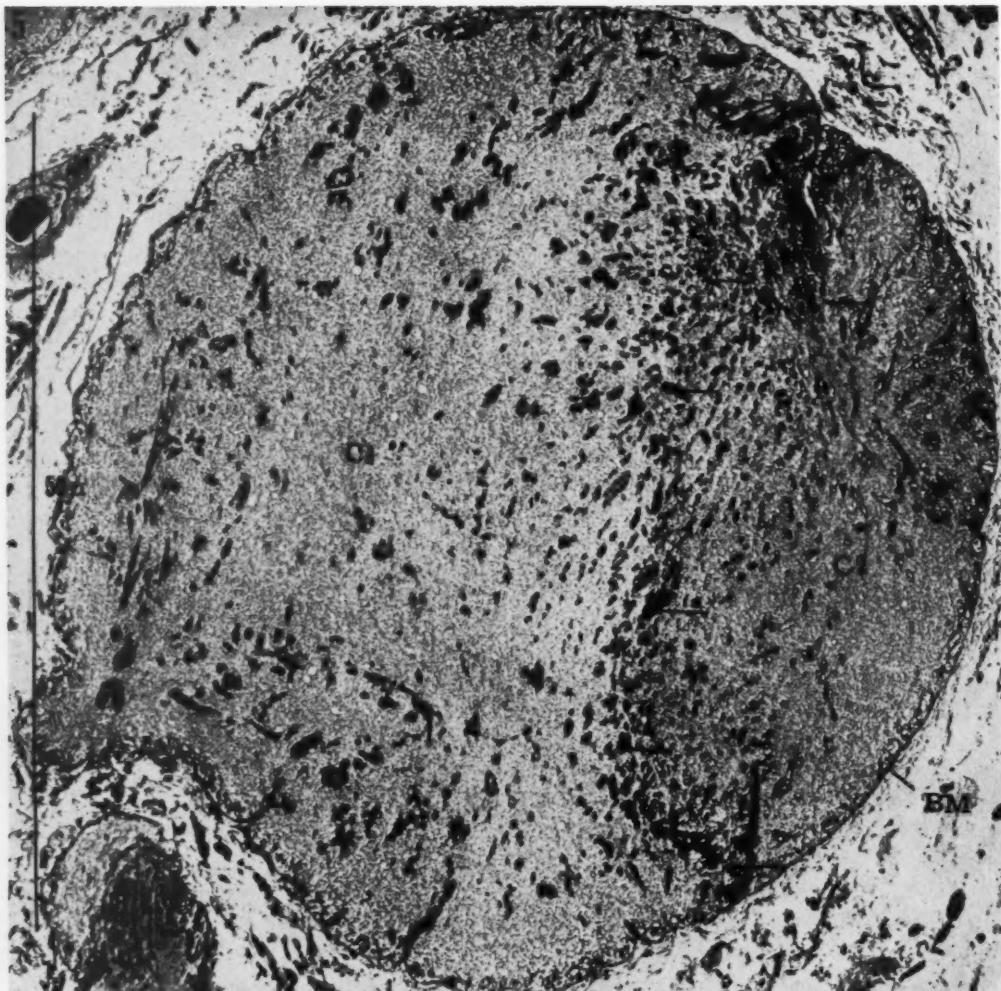


Figure 5

*Low magnification electron micrograph of a cross-sectioned thin fiber of the common bundle. The entire width is occupied by 2 cells (C1; C2). The course of the two cell membranes facing each other is indicated by the arrows. Most of the irregular dense spots represent cross-sectioned myofibrils. A basement membrane (BM) surrounds the entire fiber uninterruptedly. (X 2,500.)*

as seen with the phase-contrast microscope, the individual cells are fairly loosely arranged, and the cross-sectioned myofibrils in the interior of the cells are quite scarce. In a cross section of A-V nodal fibers (fig. 14) using the same magnification, the cells are somewhat more closely arranged and show a greater abundance of myofibrils. In longitudinal sec-

tion, the S-A node (fig. 13) shows a striking similarity to the structure of the common myocardium of the atria. The individual fibers communicate by side branches, giving rise to a meshwork known to exist in the cardiac muscle tissue itself. The nodal cells usually meet end to end, marked by the dense cross lines that represent intercalated disks,



Figure 6

Low magnification electron micrograph of a longitudinally sectioned fiber of the common bundle. At least 4 cells (C1 - C4) are seen. The overall density of the cells varies because of a variation in the finely granulated cytoplasm. The cell C4 is most dense and contains a great number of submicroscopic vesicles of unknown origin and function. The myofibrils are widely scattered but tend to become aggregated along the cell borders. Most fibers are thin (F1) but occasional thick ones (F2) occur. The nucleus (N) has a heavily stained but loosely arranged nucleolus. ( $\times 2,200$ .)



Figure 7

*Detail of 2 common bundle cells (C1; C2). The course of the delicate plasma membrane of each cell is indicated by arrows. A dense band follows the general course of the cell boundaries and becomes attached at the plasma membrane of either cell in a desmosome-like structure at or between X - X. Mitochondria (M) are spherical and display the usual fine structure of this cell organelle. Smooth-surfaced vesicles (V) of varying size with a clear center are abundant in these particular cells. The fine background granu-*

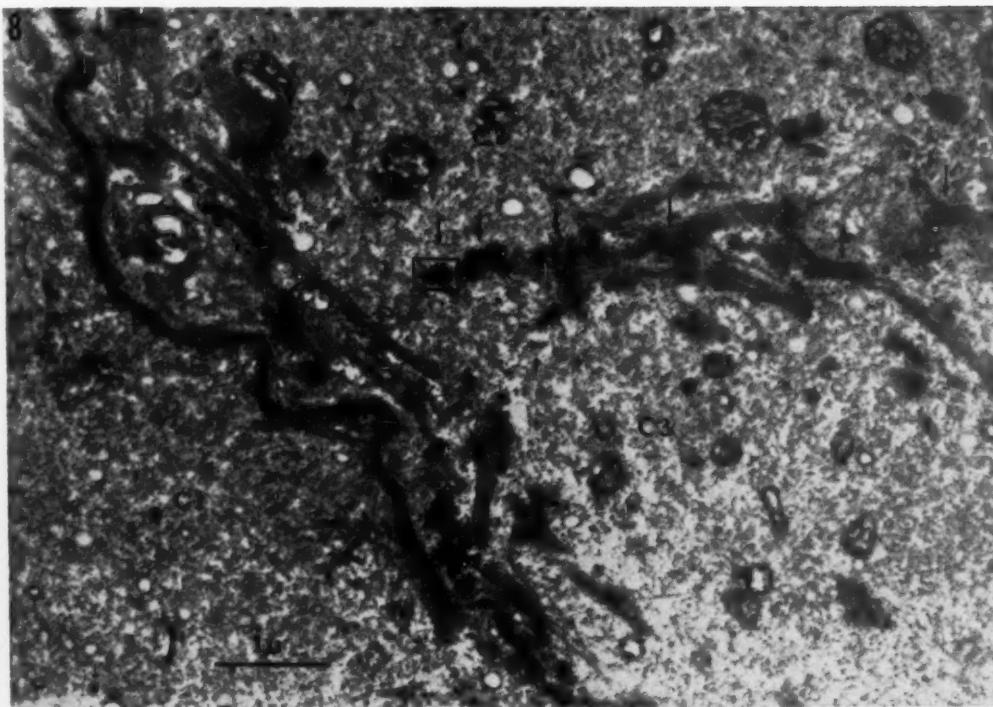


Figure 8

The junction of 3 common bundle cells (C1-C3). In C1, a long and dense cytoplasmic band can be traced, whereas this structure is not present in C2 and C3. On the other hand, several desmosomes (arrows) of varying length can be seen here. The rectangle is enlarged in figure 9A. ( $\times 18,500$ .)

similar to the structures of the common myocardium. A relatively large number of nuclei and disks are present, indicating that the nodal cells are comparatively short. It is easy to obtain a section of the S-A node where the fibers are parallel, but this is not so in the A-V node (fig. 15). Here, the fibers tend to run in many directions, a fact demonstrated by earlier investigators. The nodal cells here are joined end to end as in the S-A node, but they are longer, judging by the relatively fewer intercalated disks and nuclei.

Apart from this, the A-V nodal fibers are thicker and more densely arranged and seem to contain a larger number of myofibrils than do the cells of the S-A node.

#### Electron Microscopy

At the ultrastructural level, there is basically little difference between the cells of the S-A and the A-V nodes in the steer heart. Therefore, only the fine structure of the cells of the S-A node will be considered.

The organization of the cells of the S-A node is very much the same as that of the

larity is believed to represent glycogen. The myofibrils (F) display thin filaments that have the usual characteristics of myofilaments. The Z-bands (Z) are most prominent; the H-bands (H) with the central thin M-disk can also be seen. This particular myofibril is contracted because the light I-bands on either side of the Z-band cannot be observed. The dense cytoplasmic band that accompanies the cell borders is occasionally seen (\*) to make a connection with a Z-band. ( $\times 23,400$ .)

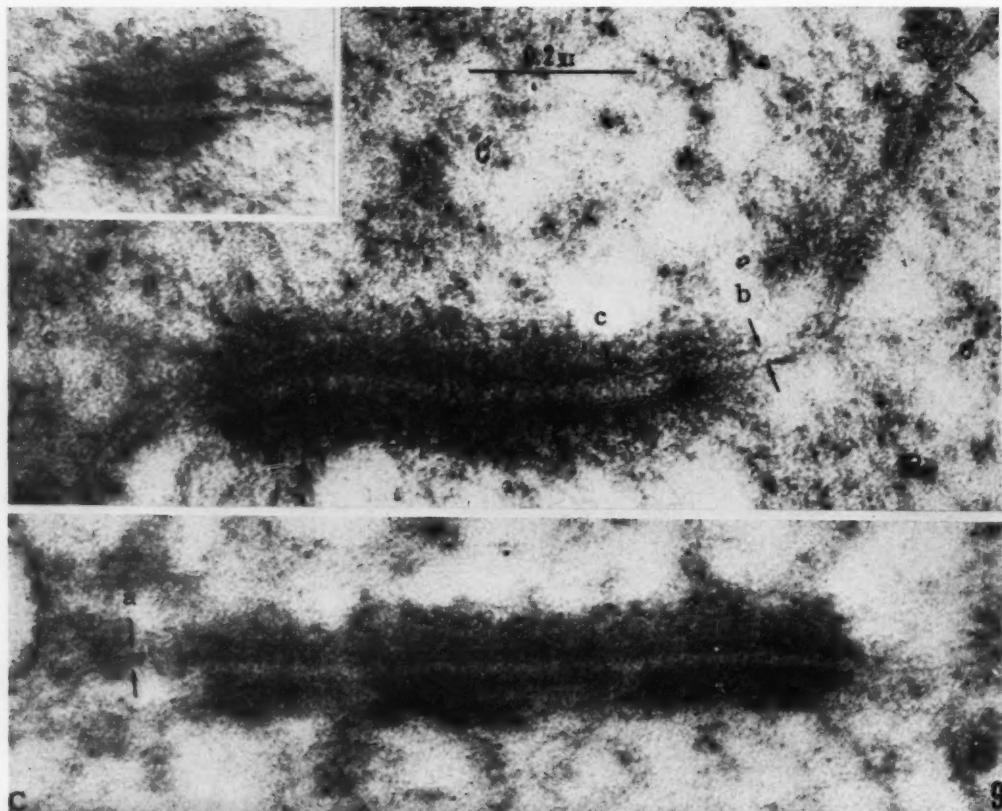


Figure 9

*Detail of the desmosomes present in the cells of the common bundle. They vary considerably in length. The plasma membranes are about 50 Å thick and separated by a 50 Å wide intercellular space (a). Closer to the desmosome (b) the intercellular space widens and reaches a width of about 175 Å within the desmosome (c). A second dense layer, parallel to the plasma membrane, is resolved within the desmosome (most clearly seen in A and C). (× 128,000.)*

common myocardium (fig. 16). Indeed, the similarity is so striking that it would be virtually impossible to tell them apart if reference could not be made to the anatomy and the histology of the tissue; then the two tissues look different according to earlier investigators<sup>27</sup> and also to our own description here. Because of the similarity between the cells of the S-A node and the common myocardium, we do not find it necessary to go into a lengthy discussion about their ultrastructure but refer the reader to earlier electron microscopic investigations of heart muscle.<sup>14, 18, 20-28, 28</sup>

However, a few points of importance in reference to the present problem will be brought up.

Each cell is enclosed by its plasma membrane, a delicate envelope about 70 Å thick. The cells vary in length and meet only end to end, with the point of contact marked by the intercalated disk. Individual cells branch and interconnect with neighboring cells to form a three dimensional meshwork of muscle fibers. The cells are also wrapped by a thin basement membrane that does not penetrate between the cells (fig. 17). Usually each cell has 1

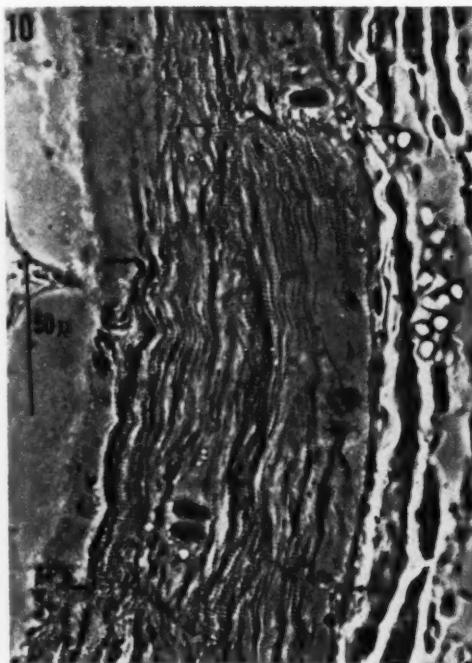


Figure 10

Phase-contrast micrograph of a longitudinally sectioned fiber of the left distal branch of the common bundle. The cells are more elongated than in the common bundle and they meet in preference end to end (arrows). They contain a fairly large number of cross-striated myofibrils. (Osmium tetroxide fixation; unstained plastic section;  $\times 500$ .)

nucleus but occasionally 2 nuclei can be seen in the same cell. Seen in a section, about 75 per cent of the cell is occupied by myofibrils, characterized by the well-known pattern of cross striations. Mitochondria are distributed along the plasma membrane and in between the myofibrils.

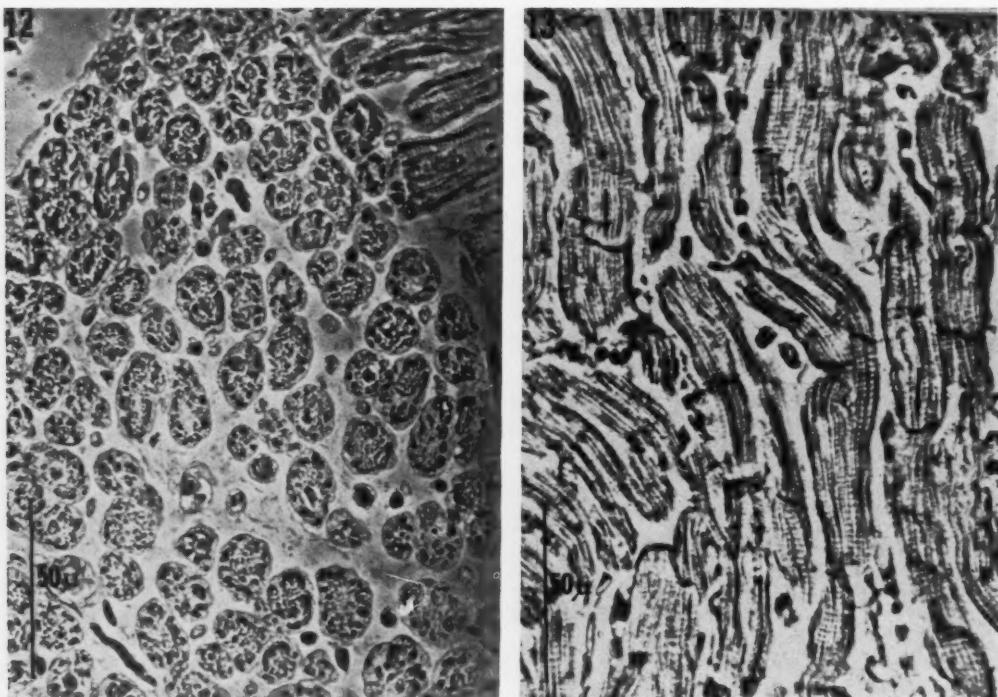
The *intercalated disk* runs across the entire width of the nodal fibers (fig. 18). For many years, it was believed that the common myocardium consisted of cells without true cell borders, thus forming a *syneytium*. With the aid of the electron microscope,<sup>14, 18, 20-23, 28</sup> however, it became clear that the intercalated disk represents the junction of cardiac cells, a fact that once and for all overthrew the concept of myocardial *syneytium*.<sup>27, 29, 30</sup> The



Figure 11

Light micrograph of the sino-atrial node (SA) and the myocardium (Y). The size of the cells is approximately identical in both instances, but the cells are more loosely arranged in the S-A node. (Formalin fixation; hematoxylin-eosin stain;  $\times 100$ .)

cells of the nodal fibers in the steer heart are joined similarly. As the myofibrils approach the intercalated disk, the individual myofilaments seem to spread slightly before they attach at the disk (fig. 18). The intercalated disk is actually composed of short dense condensations or subunits of the cytoplasm adjoining the intracellular aspect of the plasma membrane of the ends of two neighboring cells. The myofilaments that approach the end of the nodal cell then establish an anchorage in the dense subunits at the plasma membrane (fig. 19). The plasma membrane is always rather wavy at the intercalated disk. In between the dense subunits, it appears smooth and devoid of attached cytoplasmic structures. Occasionally, however, the desmosome type of cell-to-cell contact can be identified,



Figures 12 and 13

*Phase-contrast micrographs of cross-sectioned (fig. 12) and longitudinally sectioned (fig. 13) fibers of the S-A node of the steer heart. The fibers are loosely arranged with a fair number of myofibrils. Some of the intercalated disks are indicated by arrows. (Osmium tetroxide fixation; unstained plastic sections;  $\times 500$ .)*

but these structures do not receive myofibrils. This structure has been found and carefully analyzed in the common myocardium of mouse and guinea-pig hearts by Sjöstrand and co-workers. It was called an "S-region" and corresponds structurally to the desmosomes described here in the cells of the common bundle of the steer heart.

#### Discussion

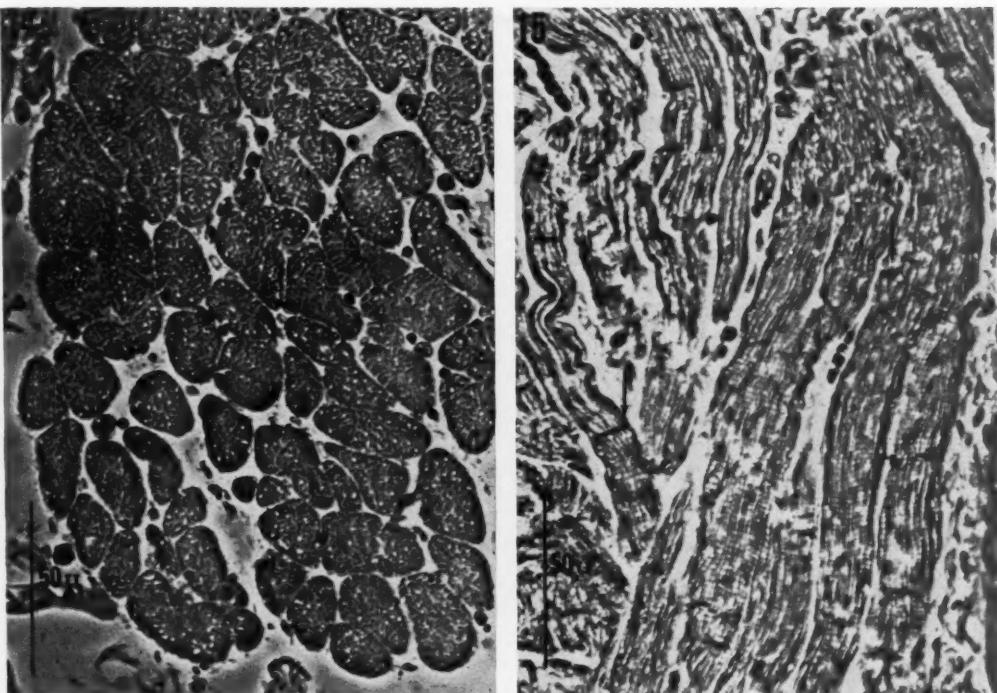
##### Anatomy

At present, there is no doubt that a specific tissue does exist in the myocardium of the steer heart. The dissections by del Missier et al.<sup>7</sup> demonstrated that it is fairly easy to show its gross anatomic distribution not only in the heart of the steer but also in those of man, dogs, pigs, and sheep. The histologic appearance of the specific tissue was most

convincingly analyzed by Blair and Davies<sup>27</sup> in the bovine heart. Our light and phase-contrast microscopic studies essentially confirm those of Blair and Davies. The remarkable similarity between the structure of the common myocardium and the S-A and A-V nodes should again be stressed. The only gross difference seems to be the comparatively loose arrangement of the fibers of the nodes with the interspaces occupied by connective-tissue elements.

##### Electron Microscopy

Earlier investigations of the specific tissue by means of electron microscopy have dealt with this structure in the myocardium of the sheep heart. Muir,<sup>6</sup> who made the first electron microscopic study of the specific tissue, analyzed the distal branches of the common



Figures 14 and 15

Phase-contrast micrographs of cross-sectioned (fig. 14) and longitudinally sectioned (fig. 15) fibers of the A-V node of the steer heart. The fibers are arranged more densely in the A-V node than in the S-A node and they also contain a greater number of myofibrils. The cells are longer in the A-V node and therefore display a smaller number of intercalated disks (arrows). (Osmium tetroxide fixation; unstained plastic sections;  $\times 500$ .)

bundle, whereas Caesar et al.<sup>5</sup> concerned themselves with the bundle of His and the false tendons. These works give an accurate account of the fine structure of the specific tissue cells in the sheep heart, which for the most part conforms with the present analysis of the steer heart. The work by Muir,<sup>6</sup> in particular, is most elucidating. It demonstrates clearly the difference between the cells of the common myocardium and those of the specific tissue and, in our opinion, describes the cell-to-cell relationship more clearly than does that of Caesar et al.<sup>5</sup>

However, there seem to be certain differences at the ultrastructural level between the cells of the sheep and of steer specific tissue. These may be of minor importance

in relation to the function of this tissue, but are sufficiently pronounced to justify a short discussion of their significance. The scattered myofilaments that Muir<sup>6</sup> found and called "Purkinje fibrils" do not seem to occur in our material. Loosely arranged myofibrils do occur but without exception they display the normal bandings that the "Purkinje fibrils" of the sheep seem to lack. The significance of these findings cannot be pointed out until the specific tissues of humans and several other animals have been investigated.

Another feature is the occurrence of submicroscopic granules most of which Muir<sup>6</sup> and also Caesar et al.<sup>5</sup> interpret as representing ribonucleoprotein particles similar in nature to those demonstrated in the exocrine

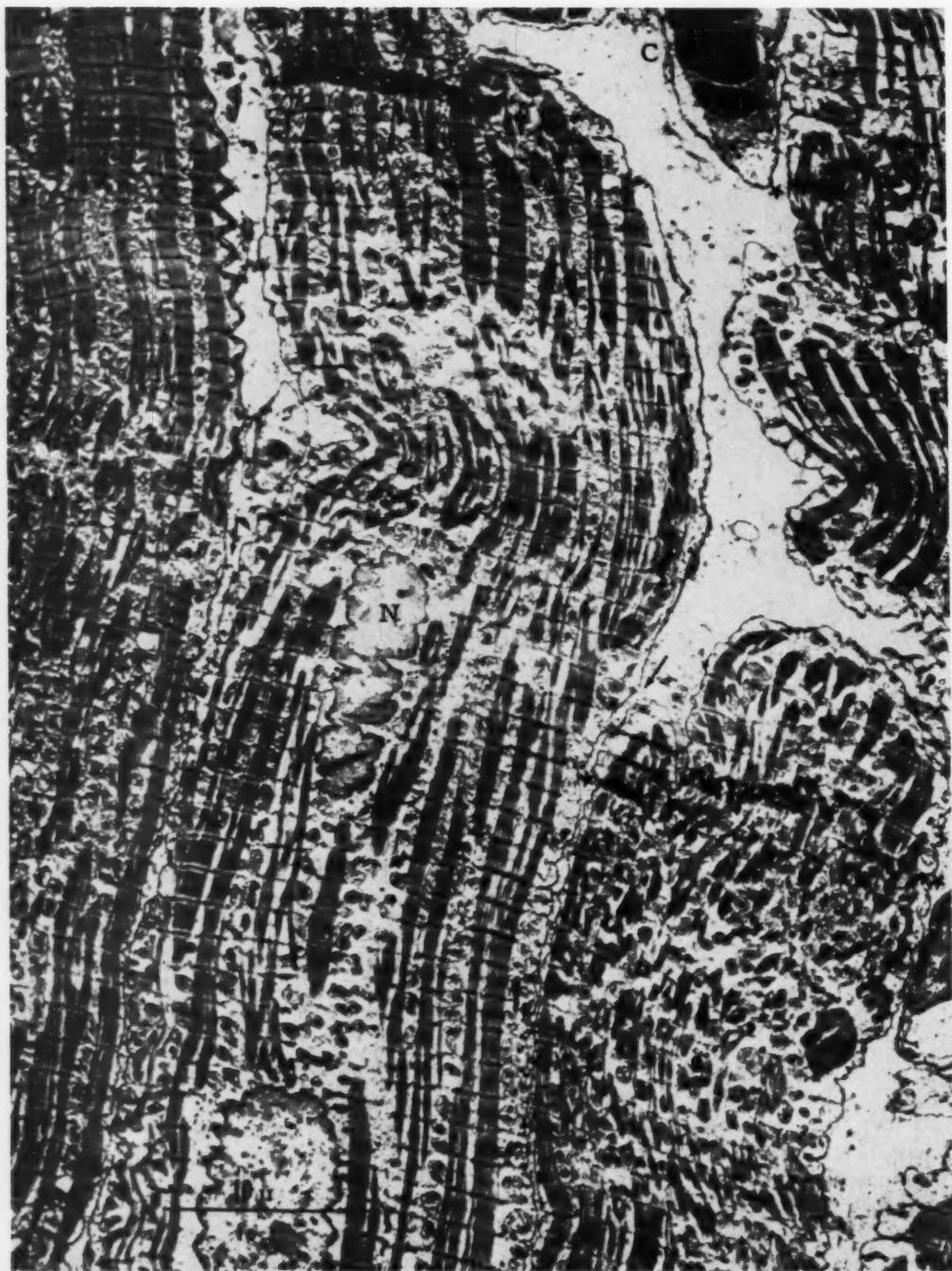


Figure 16 (See legend on opposite page)

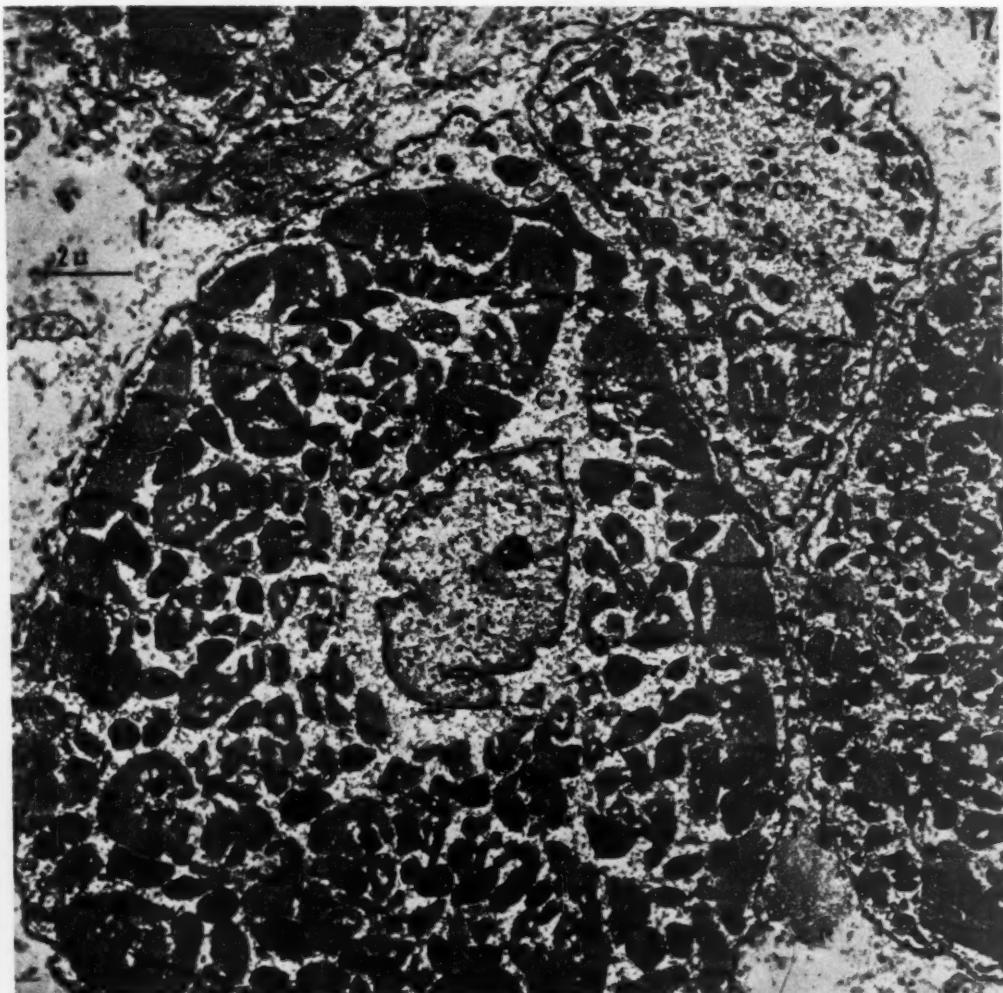


Figure 17

*Cross section of 3 fibers (C1 - C3) of the S-A node. The larger of the cells is sectioned through the nucleus (N). It is obvious that at least two-thirds of the fiber area is occupied by myofibrils. (× 7,900.)*

Figure 16

*Low magnification electron micrograph of longitudinally sectioned fibers of the S-A node. The central muscle fiber has 2 nuclei (N) and is, like the neighboring ones, enveloped by a basement membrane, which gives the surface of the fibers a scalloped appearance. Although the fibers sometimes are closely apposed, there is always an interstitial space (arrows) in which capillaries (C) are also seen. The myofibrils with their cross striations are numerous. The intercalated disks (★—★) represent true intercellular contacts without intervening basement membrane. (× 3,400.)*



Figure 18

*Intercalated-disk area of a longitudinally sectioned fiber of the S-A node. The disk runs across the entire width of the fiber (arrows). The myofilaments of each myofibril (F) seem to become more loosely arranged before they anchor at the dense structure of the intercalated disk. (X 10,500.)*

cells of the pancreas by Palade.<sup>31</sup> In our material we cannot confirm that the outer component of the nuclear membrane is studded with these granules; neither can we find vesicles provided with these submicroscopic particles. On the contrary, we find these particular membranes to be smooth and conclude that neither structure represents part of a rough-surfaced endoplasmic reticulum.<sup>32</sup> The vesiculation at the intercalated disks and the large vacuoles, which supposedly are derived from retraction of the plasma membranes as suggested by Caesar et al.,<sup>5</sup> have not been recorded in the steer heart. This may be a species difference but could also be explained by poor fixation of the material analyzed by Caesar et al. Again, the ultimate solution to this problem has to await further analyses of this tissue in other animals.

#### Functional Considerations

From a functional point of view, it seems of greatest importance to compare the two different types of cellular attachments that are encountered in the various parts of the conducting system of the steer heart. In the S-A and A-V nodes, the intercalated-disk type of the common myocardium prevails, although the desmosome type of structure may also be found to a lesser degree in between the dense subunits of the intercalated disk. In the A-V bundle and its left and

right branches, including their fine ramifications, the multiple desmosome type of attachment has been found exclusively in the present study of the specific tissue of the steer heart (fig. 19).

The function of these two types of structure is obviously to secure the mutual attachment of cells, a conclusion reached by comparison with other instances in which an abundance of desmosomes undoubtedly serves this purpose, e.g., in the epidermis.<sup>24-26</sup> Ontogenetically, it is of interest to recall that Muir<sup>28</sup> has demonstrated by electron microscopy that the multiple desmosome type is present in the common myocardium of the rabbit in embryos from 18 days after coitus to birth. Later on, the adult type is developed, with the characteristic wavy course of the cell boundary within the intercalated disk. This seems to indicate that in the steer heart the cells of the common bundle and its branches with the multiple desmosome type may represent a tissue that has maintained its embryologic appearance. The specific tissue may, therefore, represent the vestigial remnant of the first vascular tube, phylogenetically it would be the oldest tissue in the heart and ontogenetically the first to appear. There may also be another function pertaining to the multiple desmosomes versus the intercalated disks. Sjöstrand and co-

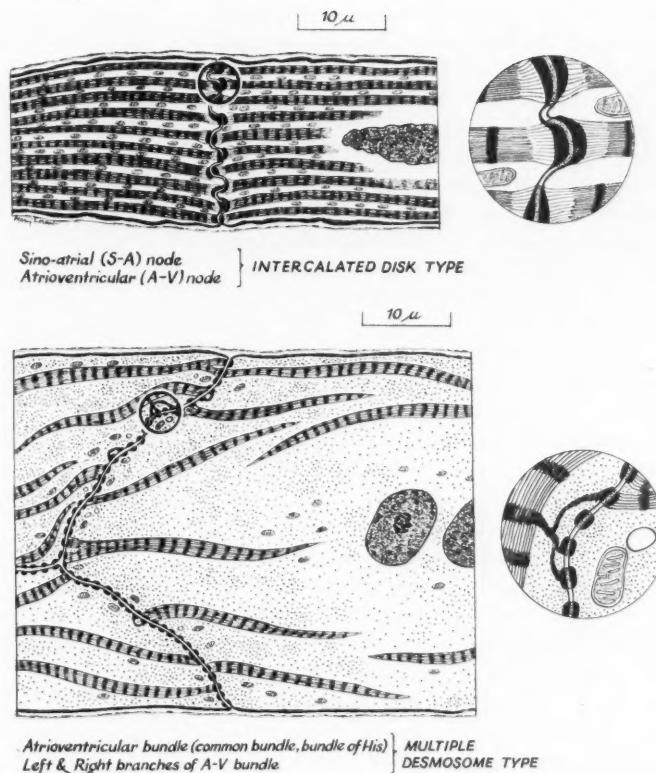


Figure 19

Schematic representation of the cellular contacts as seen in the various parts of the impulse-conducting system of the steer heart.

workers<sup>20-22</sup> have suggested that the specialized regions they found in the common myocardium between the disk regions and that closely resemble our desmosomes may represent areas with a lower ohmic resistance and may form paths with a great safety factor for conduction across the cell junction. Our findings of a multitude of desmosomes in the lower regions of the conducting system, where certainly the impulse travels so much faster than in the common myocardium, seem to support this hypothesis.

Finally, we may, in summing up, compare the histologic and ultrastructural appearance of the various parts of the conducting system? From our studies, it is fairly clear that the passage of the stimulus in any part of the conducting system could occur by a wave of muscle contraction. Any electrical effects produced by this wave of contraction are undoubtedly the results of previously

activated, enzymatically catalyzed chemical processes. Bourne<sup>33</sup> has reported the presence of alkaline phosphatase in the intercalated disks of the common myocardium, and this enzyme may well be present in the disks of the specific tissue also. Succinic dehydrogenase<sup>33, 34</sup> and cholinesterase<sup>35</sup> are both concentrated along the borders of the specific tissue cells, presumably in the mitochondria but possibly also located in the small vesicles described here. If the transmission of the impulse is facilitated by the intercalated disks and the desmosomes and if the impulse is mediated by enzymes located in or near these structures, then one would expect it to travel faster across a border where these structures are frequent. We do not know the rate of conduction in the S-A node itself, but it is 1,000 mm. per second in the atrial muscle and the number of intercalated disks per 40,000 square microns in a longitudinal

section of the atrial muscle is about equal to that of the S-A node (fig. 13), possibly indicating that the rate of conduction is roughly the same. The rate of conduction in the A-V node is about 200 mm. per second and in the ventricular muscle 300 to 500 mm. per second. Again, there is a close structural resemblance between the A-V node and the ventricular muscle. Furthermore, the slower rate, as compared with the atrium, may be explained by the smaller number of intercalated disks and the larger size of the cells. Once the impulse reaches the tissue of the common bundle, the rate of conduction increases to 3,000 to 5,000 mm. per second, possibly explained by the great number of desmosomes and the large area they represent per square unit of cell surface.

A purely mechanical point of view could also be introduced at this moment. The structure of the S-A node, the A-V node, and the common myocardium of the atria and ventricles closely resembles that of a fisherman's net with a third dimension added, whereas the structure of the common bundle and its branches reminds one of a set of strings connected here and there along its course (fig. 1). A pull at one corner of a net would spread in all the directions of the net's meshes, thus delaying the pull to be felt at the opposite corner of the net. A pull at one end of a set of strings would be felt much faster at the opposite end because no spreading in a wide three dimensional meshwork is involved.

Of course, many other factors would have to be taken into consideration<sup>36</sup> in explaining the high rate of conductivity of the cells of the specific tissue as well as in explaining the magic ability of the entire conducting system to originate autonomously a stimulus for contraction. Here, such factors as the high content of glycogen<sup>6</sup> as well as of sphingomyelin<sup>37</sup> probably play an important role.

#### Acknowledgment

We are indebted to Dr. A. A. Angrist for help and advice in dissecting the hearts. We also gratefully acknowledge the skillful technical assistance

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## Blood Capillaries of the Heart and Other Organs

By GEORGE E. PALADE, M.D.

The article is a review of work recently carried out on blood capillaries by the author in collaboration with Drs. M. G. Farquhar, G. Majno, and S. L. Wissig.

It reviews the morphology of these vessels at the electron-microscope level and confirms the existence of at least 3 distinct types of blood capillaries in small laboratory mammals. It shows that the capillary wall consists of 3 concentric layers (endothelium, basement membrane, and adventitia), and indicates that the basement membrane forms a continuous layer in all capillaries so far studied.

Experiments in which colloidal gold particles were used as a tracer have shown that, in capillaries with a continuous endothelium (muscle capillaries), the particles are transported across it by "pinocytic" vesicles. At the end of this step they must still transverse the basement membrane.

Experiments on glomerular capillaries, which typically have a discontinuous endothelium, were carried out on normal and nephrotic rats using ferritin as a tracer. By its accumulation on the luminal side of the basement membrane, the ferritin has identified this layer as the main filtration barrier.

A similar function of the basement membrane was demonstrated in muscle venules and venous capillaries by experiments in which the endothelium was rendered discontinuous by local treatment with histamine and serotonin.

HERE ARE, I believe, a good number of reasons for a renewed interest in problems of capillary permeability. To begin with, it is clear that we are dealing with a basic process in the physiology of metazoa. In these complex organisms, the life of the multitude of cells in the intimacy of tissue depends, in ultimate analysis, on the ample and continuous exchanges that take place across the wall of capillary vessels between the blood plasma on one side, and the interstitial fluid on the other. To continue, many pathologic conditions can be traced back to circulatory disturbances in general, and to variations in capillary permeability in particular, an outstanding example being the inflammatory process. And to finish, the mechanisms involved in the exchange of large quantities of water and solutes across the capillary wall are still poorly understood.

Studies on the structural aspects of the problem, carried out over many years by light

microscopy, have revealed only a few general morphologic features that facilitate the exchanges: one could list under this heading the small diameter of the vessels, their organization into a tight meshwork that ensures a high surface-to-volume ratio for the circulating blood, and finally the extreme tenuity of the capillary wall. A description of these features, together with an attempt to quantitate them in terms of capillary volume and capillary surface per unit volume in various tissues, figured prominently—for instance—in August Krogh's book "The Anatomy and Physiology of Capillaries," which summarized what was known in 1928 about the morphology and physiology of blood capillaries.<sup>1</sup> In this book, which had considerable influence on the subsequent development of the field, Krogh assumed that the capillary wall consisted only of a layer of endothelial cells\* but, aside from stressing their extreme thinness, did not further inquire into structural devices directly

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\*The existence of a second layer, called basement membrane ("Grundhautchen"), was described, however, by many histologists. See, for instance, Benninghoff.<sup>2</sup>

involved in capillary permeability. With this in mind, it would be fair to say that we inherited from our light-microscope predecessors a good knowledge of the general layout of the capillary vessels, but practically no knowledge regarding their fine structure and especially the structural details involved in capillary permeability.

By comparison, the physiologic aspects of the problem have been more thoroughly investigated and seem to be better understood. Ever since Starling's 1896 hypothesis,<sup>3</sup> it has been assumed that the force that drives the fluid out of or into the capillary vessels is the difference between the hydrostatic and osmotic pressures of the blood plasma.<sup>†</sup> More recently, however, it has been realized that exchanges that operate on this basis are rather modest (a few per cent of the total) and that the mechanism in question may be more important for maintaining the blood volume than for carrying through adequate exchanges between the blood and the tissues. In this respect it is generally agreed at present that diffusion plays the major role, but difficulties are encountered when physiologic results are interpreted in structural terms. Pappenheimer and his collaborators,<sup>4, 5</sup> for instance, conclude that the permeability characteristics of the capillary wall could be explained by assuming that the wall is a rigid partition provided with patent permanent pores of  $\sim 60 \text{ \AA}$  effective diameter; whereas Chinard and his colleagues<sup>6</sup> believe that the wall behaves like a laminar gel permeated by a continuous aqueous phase—fibrils and interfibrillar spaces in the gel being of molecular dimensions.

It should be pointed out that in all these structural extrapolations it is assumed that the capillary wall consists of a single cellular layer, the endothelium. In Pappenheimer's formulation, the postulated pores occupy a small part of the wall surface ( $\sim 0.1$  per

cent) and are presumably located along the cell junctions, cutting—so to say—through the intercellular cement, a hypothetical substance that fills all the intercellular spaces of the endothelium. The hypothesis according to which the exchanges between blood and tissues are carried through these cement-filled spaces was originally advanced by Chambers and Zweifach.<sup>7</sup> Before ending this short review of the physiologic aspects of the problem, I should add that the capillary wall is more permeable to lipid than to water-soluble substances. For this reason Pappenheimer<sup>4</sup> and Renkin and Pappenheimer<sup>5</sup> have actually postulated a double pathway across the wall: via the pores for water and solutes, and via the cells proper for lipid soluble substances.

#### The Fine Structure of Blood Capillaries

This was the general state of our knowledge before the electron microscope was used to investigate the structural aspects of the problem. The reinvestigation has already provided a large body of information and has established firmly at least two points.<sup>cf. 8, 9</sup>

*First:* The capillary wall is a multilayered structure. In addition to a cellular endothelial layer, which could be described as an internal tunic, it comprises an acellular layer—the basement membrane (middle tunic)—and an outer, discontinuous stratum of cells and fibers that constitute an adventitial tunic.

*Second:* Although similar in their general construction, capillary vessels differ constantly and characteristically in their structural details from tissue to tissue, or, rather, from groups of tissues to groups of tissues. The differences affect primarily the cellular layers, the endothelial and the adventitial, which could be discontinuous or even absent, whereas the basement membrane generally persists as an uninterrupted layer. This continuity of the middle tunic, or basement membrane, emerges then as a common structural feature for practically all types of capillary vessels so far examined.\*

<sup>†</sup>Actually the algebraic sum of the following terms: hydrostatic pressure of the blood plasma, osmotic pressure of same, hydrostatic pressure of interstitial fluid ("tissue pressure"), and osmotic pressure of same.

\*See, however, Bennett et al.<sup>1</sup> and Wood<sup>10</sup> on the problem of hepatic blood sinuses.

With this preparation, we can start reviewing the electron microscopic evidence.\* Figure 2 illustrates the type of capillary encountered in a skeletal muscle and in other tissues of the soma but also present in certain viscera, e.g., the myocardium and the smooth muscle of the digestive and reproductive tract. It is characterized by a continuous endothelium, 0.5 to 0.2  $\mu$  thick, and a continuous basement membrane. The endothelial cells are extremely flat but otherwise similar in organization to other animal cells. They possess a nucleus, a centrosphere region with 2 centrioles and a few small piles of smooth-surfaced cisternae,

\*The morphology of blood capillaries was studied in a number of small mammals (rats, hamsters, guinea pigs, rabbits) by electron microscopy, using tissue specimens fixed in  $\text{OsO}_4$  (buffered at pH 7.4 to 7.6) and embedded in methacrylate.

The experimental work was carried out exclusively on rats, using the same preparative procedures for electron microscopy.

a more or less developed endoplasmic reticulum, ribonucleoprotein (RNP) particles, mitochondria, and a cytoplasmic matrix in which fine fibrils can sometimes be detected. The only distinguishing, although not unique, feature is represented by a large number of vesicles<sup>11, 12</sup> located immediately below the cell membrane along both the blood and the tissue fronts of the cell (fig. 1). A closer examination reveals that some of these vesicles are open to the cell surface, while others are closed. Those open could be described as invaginations of the cell membrane, since their limiting membrane is continuous with the cell membrane. In this situation their content is also continuous with the extracellular fluid: blood plasma on one front of the cell and interstitial fluid on the other. Between closed and open vesicles a whole spectrum of possible intermediates is encountered. Grazing sections show the vesicles are quite numerous—120 to 140 per  $\mu^2$ —on the luminal as well as on the

#### Explanation of Plates

All figures represent electron micrographs of rat blood capillaries. The corresponding tissues were fixed in osmium tetroxide and embedded in methacrylate. The sections were stained with lead hydroxide and "sandwiched" with carbon or formvar films before examination. General Abbreviations: BM, basement membrane; EN, endothelium; EP, epithelium; L, capillary lumen.

FIGURE 1

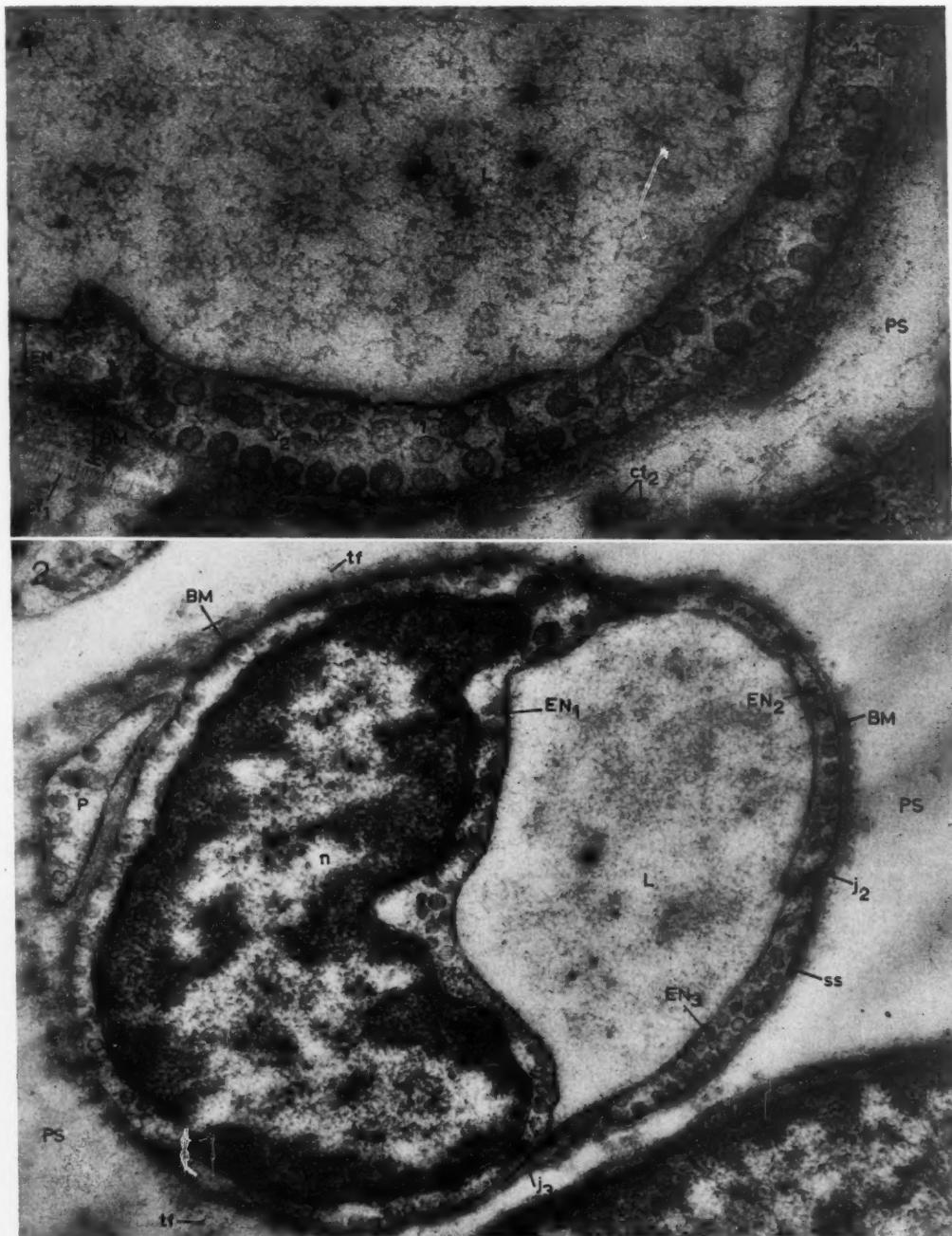
Wall of blood capillary in a skeletal muscle (rat). The lumen, which contains precipitated plasma proteins, is marked L and the pericapillary space PS. The capillary wall consists of an inner or endothelial tunic (EN), a middle tunic or basement membrane (BM), and an adventitial tunic represented here by a few collagen fibrils seen in longitudinal ( $cf_1$ ) or transverse ( $cf_2$ ) section. Small vesicles in the cytoplasm of the endothelial cell appear aligned behind the luminal and tissue fronts of the cell. Some of them ( $v_1$ ) are open to the cell surface and could be described as invaginations of the cell membrane; others ( $v_2$ ) are closed and appear located deeper in the cytoplasm. In this case, the number of vesicles is greater on the tissue than on the luminal front of the endothelium.  $\times 73,000$ .

FIGURE 2

General view of a transversely sectioned blood capillary in a skeletal muscle (rat). The lumen is marked L, and the pericapillary spaces PS. Parts of 3 endothelial cells, EN<sub>1</sub>, EN<sub>2</sub>, EN<sub>3</sub>, form the inner tunic at this level; their junctions appear at  $j_1$ ,  $j_2$ , and  $j_3$ . The nucleus of one of the endothelial cells can be seen at n.

The middle tunic or basement membrane is marked BM. It is relatively well outlined toward the endothelium, from which it is separated by a shallow subendothelial space (ss), and frays into distinct fibrils (tf) towards the pericapillary spaces.

The adventitial tunic is represented by part of a pericyte (P) characteristically enwrapped between 2 leaflets of the basement membrane.  $\times 35,000$ .



Figures 1 and 2 (See legends on opposite page)

tissue front of the cell (fig. 3), and a few simple computations indicate that the vesicles represent a considerable amount of membranous material: about  $2 \mu^2$  of membrane behind each  $\mu^2$  of cell front, and account for a sizeable part of the total volume of the cell:  $\sim 1/3$ .

The spectrum of appearances encountered could be explained by assuming that the vesicles are formed by invaginations of the cell membrane that are pinched off and become closed elements in the cytoplasm, carrying an imprisoned droplet of extracellular fluid. A reverse process would evidently produce the same series of appearances, but this time the sequence would start with a fluid-filled vesicle in the cytoplasm, which moves to the surface, where its membrane coalesces with the cell membrane, and where its content is discharged in the extracellular medium by the orifice created at the site of the coalescence. If the 2 processes are combined, the vesicles could transport fluid from one front of the cell to the other in small, more or less equal portions or quanta. The diameter of such a pocket is  $\sim 650$  to  $750 \text{ \AA}$  and its volume  $\sim 100,000 \text{ m} \mu^3$  to  $\sim 180,000 \text{ m} \mu^3$ .

This hypothesis has been tested experimentally but, before presenting the appertaining results, I should like to close the morphologic inquiry by reviewing information obtained

on the cell junctions and on the basement membrane in this type of capillary. At the level of the cell junctions (fig. 4), there is a narrow intercellular space  $\sim 100 \text{ \AA}$  that separates 2 symmetric densifications of the apposed cell membranes. The latter are sometimes backed by a condensation of the subjacent cytoplasmic matrix. What seems to be important is the fact that the narrow intercellular gap is occupied by a material of moderate density frequently condensed into a denser intermediary layer or lamina. In other words, the narrow intercellular gap is not an unobstructed passage from the lumen to the pericapillary spaces. The material in the gap does not represent, however, the cement substance postulated by light-microscope studies. That cement was supposedly characterized by its ability to reduce silver ions to metal and become impregnated by it. In electron microscopy the silver deposits appear spread over a broad band centered on the junction, but preferentially concentrated within the zones of densification of the adjacent cytoplasm.<sup>13</sup> The presence or absence of pores in the intercellular spaces will be discussed later.

The second layer, the middle tunic, of the capillary wall is represented by the basement membrane, which appears as a continuous

FIGURE 3

*Grazing section of a blood capillary of the myocardium (rat) showing the large number and irregular distribution of vesicles ( $v_1, v_2$ ) on the tissue front of the endothelium. The "stoma" of some of these vesicles, i.e., their opening to the cell surface, shows clearly as a light, circular area ( $v_2$ ).*

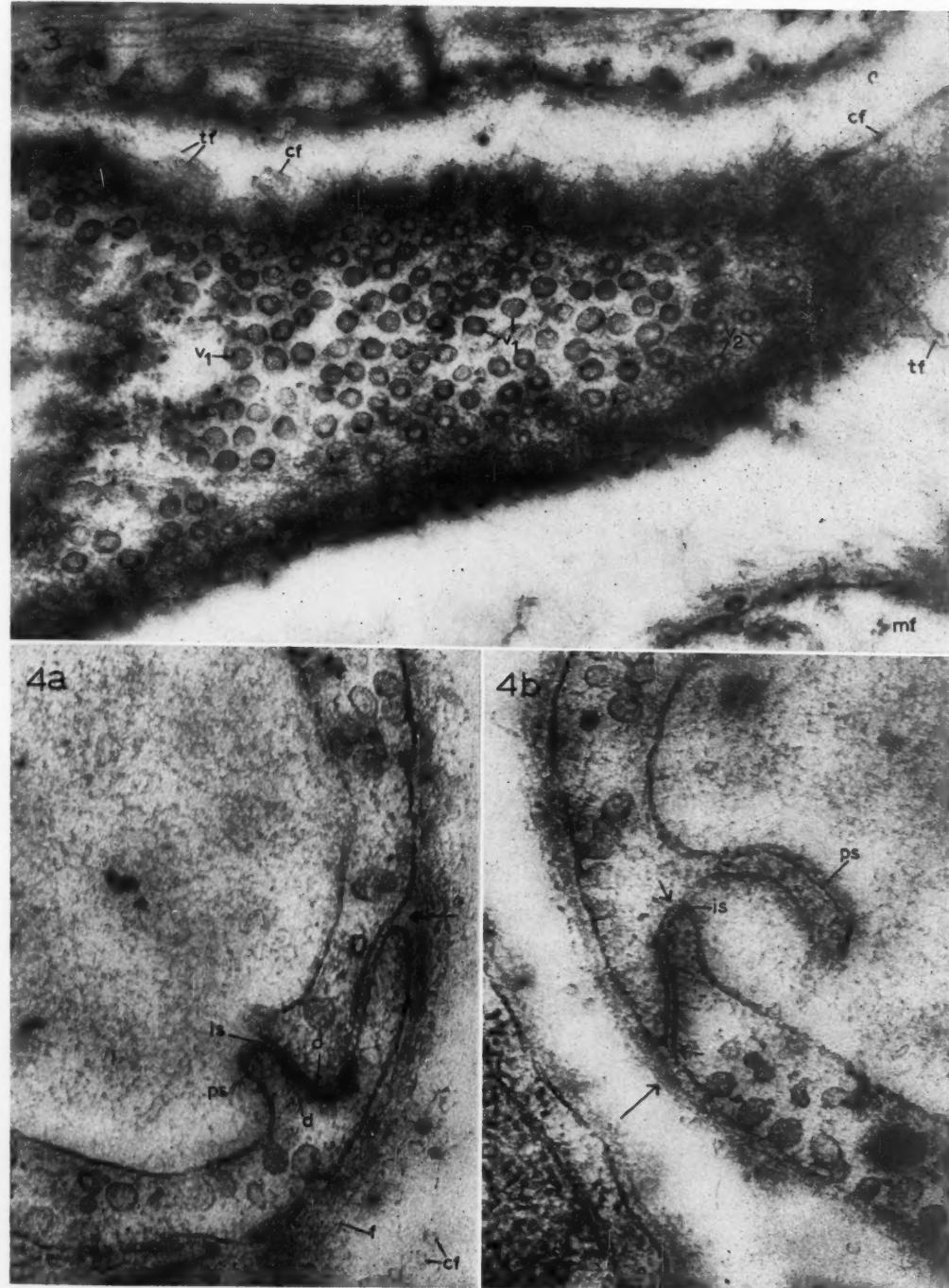
*The basement membrane appears as a densely matted felt of fine fibrils (f), with sparse, slightly thicker fibrils in its peripheral layers (tf). A few collagen fibrils of the adventitia are visible at cf. Adjacent muscle fibers are marked mf.  $\times 55,000$ .*

FIGURE 4, a and b

*Epithelial cell junctions in blood capillaries of skeletal muscle (rat). The thickening of the apposed cell membranes is visible in Fig. 4b in between the short arrows. The companion densification of the subjacent cytoplasm shows more clearly in Fig. 4a at d. The intercellular space (is) is occupied by a material of higher density than that filling the pericapillary spaces.*

*Note in both cases the oblique or sinusoidal course of the junctions, the pseudopodia (ps) that flank them on the luminal front, and the fact that the basement membrane passes without interruption or infolding over the junction (long arrows). The fibrillar texture of the basement membrane can be distinguished at f. Collagen fibrils are marked cf.  $\times 71,000$ .*

Figures 3 and 4



Figures 3 and 4 (See legends on opposite page)

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coat of moderately dense material 200 to 500 Å in thickness. The limits of the coat are rather sharp toward the endothelium, from which it is separated by a narrow subendothelial space, and more poorly outlined toward the pericapillary spaces. In this direction the basement membrane comes in contact with various adventitial elements—collagen and elastic fibrils, and cells of varied type, pericytes, macrophages, fibroblasts, and others.

In view of the tenuity and moderate density of this basement membrane, one may wonder whether we are dealing here with an independent structural element of the wall or merely with a condensation of the ground substance of the connective tissue, which could disperse easily once the endothelial substrate is removed. The answer is found in damaged specimens which show extensive retraction of the endothelium: although left behind, the basement membrane subsists as a distinct layer; apparently it is cohesive enough to resist the various mechanical and chemical insults involved in our preparation procedures.

In specimens fixed by osmium tetroxide, the basement membrane appears as an amorphous layer of more or less homogenous density. In preparations stained by heavy metals, however, fine fibrillar elements of higher density can be demonstrated therein (fig. 5). In fact, 2 types of fibrillar elements can be recognized: one finer and tightly meshed in the inner parts of the layer; another coarser, less abundant, and less intertwined in the outer parts

of the structure. Grazing sections through the capillary wall are particularly favorable for demonstrating these fibrillar components, both of which appear to be distinct from mature collagen fibrils: they are thinner and do not show the characteristic periodic pattern of the latter. The coarser of the fibrils found in the basement membranes look morphologically similar to a special type of fibril usually encountered around elastic fibers. Needless to say, the chemical nature of all these fibrillar elements is unknown.

On the strength of this evidence we can conclude that the basement membrane is a felt of fine fibrils and that the meshes of the felt seem to be filled by another material, a matrix, which still appears amorphous at the present level of resolution.

So this is the type of construction encountered in most capillaries: between the blood plasma and the interstitial fluid in interposed a succession of barriers consisting of a continuous endothelium, a continuous basement membrane, and an adventitia that, in this case, is discontinuous enough to be negligible as a true barrier.

#### Tracer Experiments on Blood Capillaries in Striated Muscles

What is the functional role of these layers in blood-tissue exchanges? What structures participate and what mechanisms are involved in the transport of various substances across the wall?

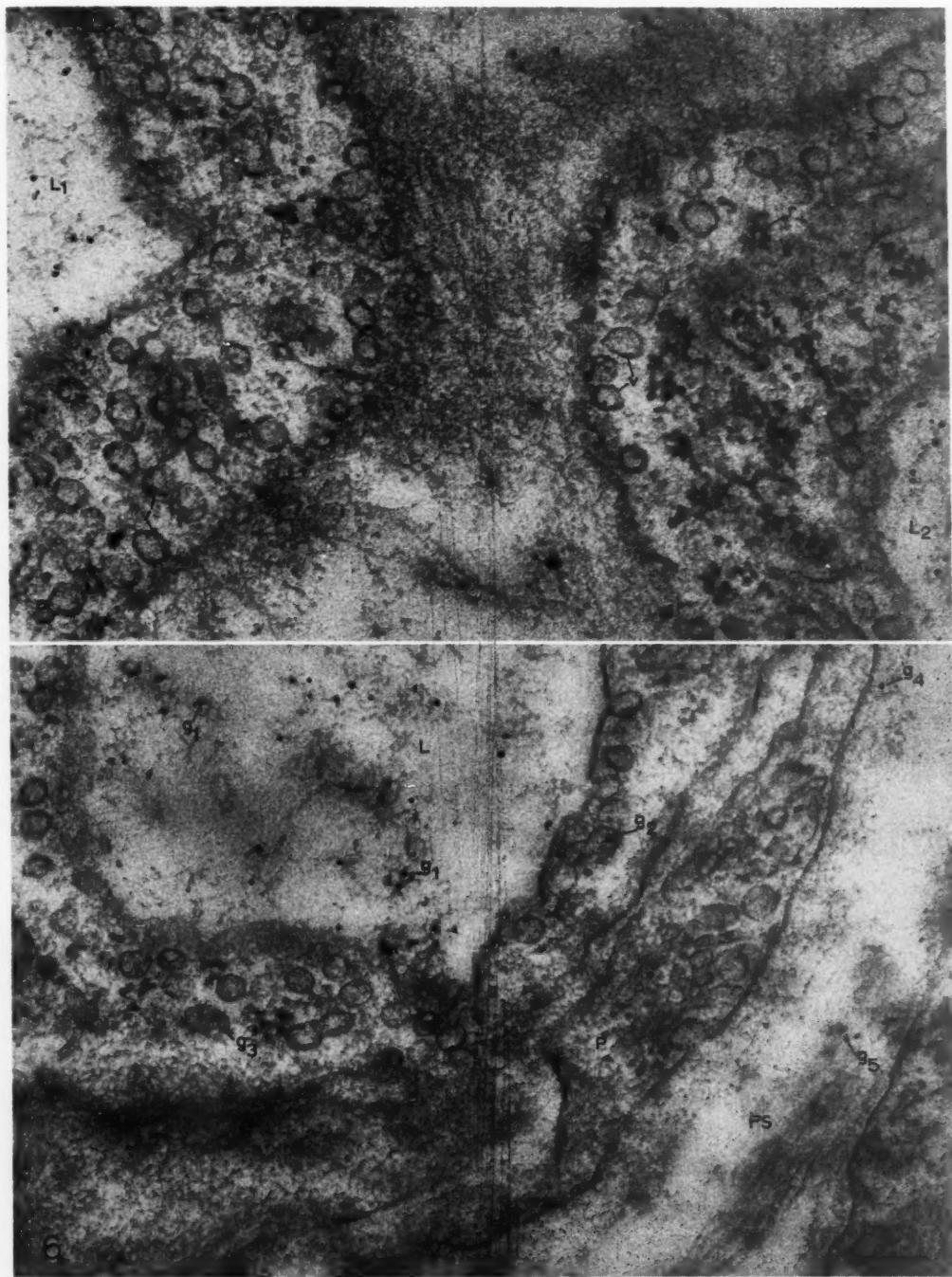
#### FIGURE 5

*Section through a bent blood capillary of the myocardium (rat). The lumen, cut in 2 places ( $L_1$ ,  $L_2$ ), is marked by colloidal gold particles. The endothelial cytoplasm contains small vesicles (v) and RNP particles (r). In the middle of the field, a grazing cut through the basement membrane exhibits its poorly resolved, felt-like texture (f).  $\times 82,000$ .*

#### Figures 5 and 6

#### FIGURE 6

*Blood capillary of the myocardium (rat) 10 minutes after an intravenous injection of colloidal gold (90 mg. in 1.5 ml.). The lumen (L) is marked by gold particles ( $g_1$ ), which are also present in the endothelium—within small vesicles ( $g_2$ ,  $g_3$ ), in the basement membrane ( $g_4$ ), and in the pericapillary spaces ( $g_5$ ). Note the sharp decrease in particle concentration from the lumen to the endothelium, and the fact that within the latter the tracer is restricted to vesicles and does not have access to the cytoplasmic matrix. At the level of this section, the endothelium is covered by the long process of a pericyte (P).  $73,000$ .*



Figures 5 and 6 (See legends on opposite page)

To find an answer to such questions we injected into the general circulation a tracer small enough to give meaningful information and dense enough to be seen individually in the electron microscope. Ferritin molecules  $\sim 100$  Å in diameter<sup>14</sup> and micelles of colloidal gold ranging from 30 to 250 Å<sup>15</sup> proved to be useful for this type of work. At intervals ranging from 2 to 60 minutes after the injection of the tracer into the general circulation, specimens for electron microscopy were collected from the heart or tongue to take advantage of the high concentration of capillary vessels in the muscle of these organs. Colloidal gold particles were found in large number in the lumen and in considerably smaller numbers in the endothelium, basement membrane, and pericapillary spaces (fig. 6). In the endothelium they were as a rule restricted to the vesicles described in the vicinity of the cell membrane, the only other structure in which they were encountered being the so-called multivesicular bodies. Only occasionally were particles found in the cytoplasmic matrix (fig. 7). Since a relatively large number of micelles were detected in the pericapillary

spaces, it can be concluded that they had been ferried across the endothelium by vesicles. In these experiments only an occasional, or no accumulation of particles was found against the basement membrane; apparently it allowed practically all micelles that crossed the endothelium to pass. After longer time intervals, one hour for instance, the situation was comparable, except that most of the particles in the pericapillary spaces were found ingested by macrophages. It should be stressed that no tracer was found at any time in the intercellular spaces of the endothelium. The results obtained with ferritin molecules were less clear cut.<sup>14</sup> Frequently the tracer was found restricted to vesicles within the endothelium, but sometimes ferritin molecules occurred freely dispersed in the cytoplasmic matrix with no indication of how they reached this location.

On the strength of the results obtained with colloidal gold, it can be concluded that the vesicles of the endothelium do function in transendothelial transport. It is clear that they ferry the marker across the endothelium and it is highly probable that, together with

FIGURE 7

*Blood capillary of the myocardium (rat) 10 minutes after an intravenous injection of colloidal gold (90 mg. in 1.5 ml.). The irregular lumen is marked by circulating particles (g<sub>1</sub>). The endothelium contains a number of similar particles enclosed singly (g<sub>2</sub>, g<sub>3</sub>) or severally (g<sub>4</sub>) into vesicles. One tracer particle (g<sub>5</sub>) appears against a slightly dense patch which could correspond to the top of a sectioned vesicle; another particle (g<sub>6</sub>) is definitely free in the cytoplasmic matrix. A few tracer particles (g<sub>7</sub>) have reached the pericapillary spaces.*

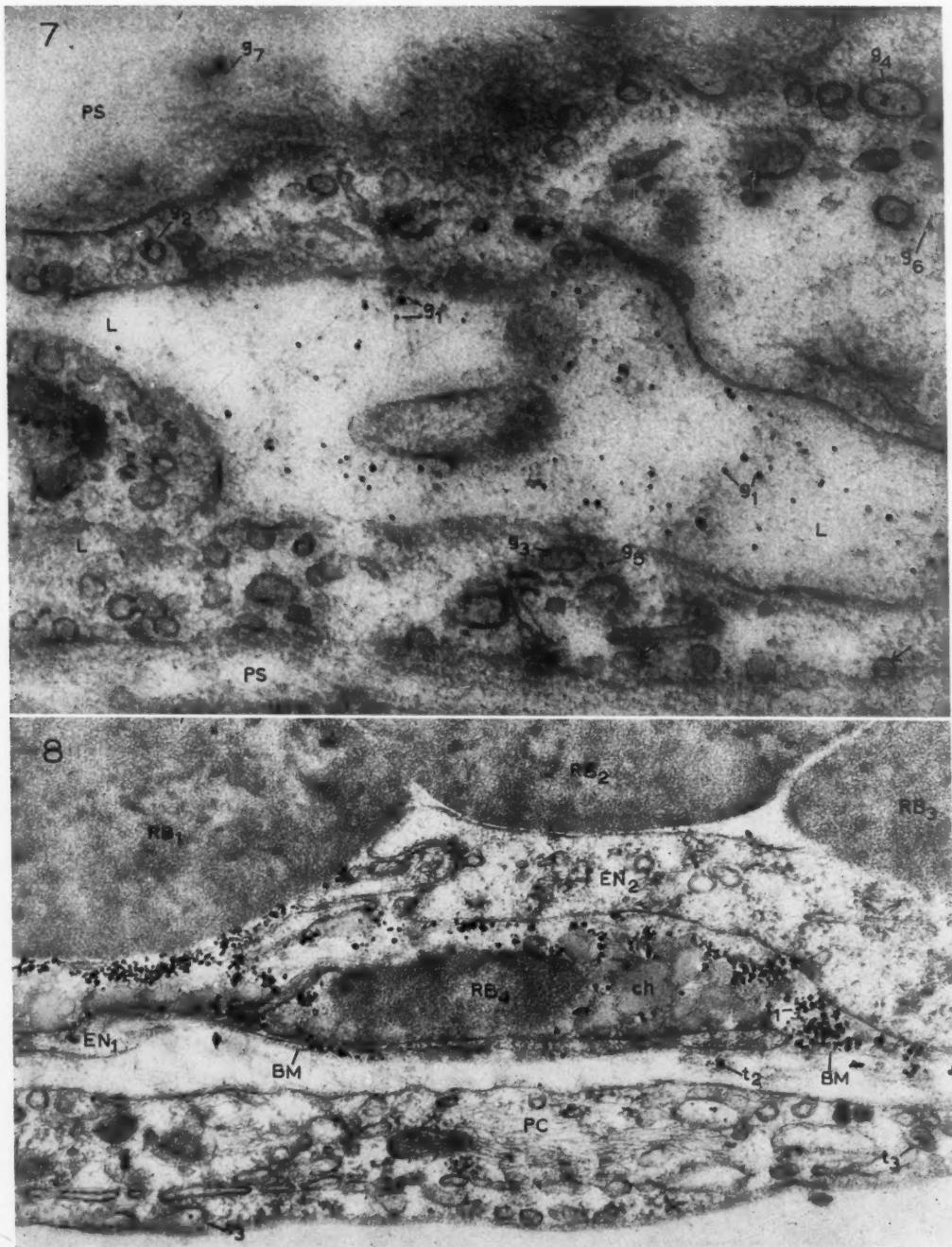
*Note that many other vesicles (arrows) contain small particles that seem to be attached to the inner surface of their membrane. Their identification as gold colloidal micelles is uncertain because their density is low and their form irregular.  $\times 77,000$ .*

FIGURE 8

*Small blood vessel (venule) in the cremaster of a rat, 2.5 minutes after a local subcutaneous injection of histamine. The tracer, colloidal mercuric sulfide, was previously injected in the general circulation.*

*The lumen is occupied by 3 erythrocytes (RB<sub>1, 2, 3</sub>). The endothelium (EN<sub>1</sub>, EN<sub>2</sub>) is discontinuous; through the gap marked by arrows, tracer particles (t<sub>1</sub>), chylomicrons (ch) and a red blood cell (RB<sub>4</sub>) have penetrated and dissected the wall up to the basement membrane (BM) which, in this case, appears particularly thin and poorly outlined. Note that most of the particles are retained by the basement membrane, and that the intramural deposit is highly concentrated, presumably as a result of water and solute escape through the basement membrane. Note also that, of the few particles which have reached the pericapillary spaces (t<sub>2</sub>), some have already been incorporated (t<sub>3</sub>) by a phagocytic element (PC).  $\times 46,000$ .*

Figures 7 and 8



Figures 7 and 8 (See legends on opposite page)

the marker, they transport water and solutes, since there is room enough for a few hundred thousand molecules of corresponding size, in addition to the tracer particle, in each vesicle of 650 Å diameter. If this modality of transport seems to be well established by our experiments, it should be pointed out that its relative importance in the overall exchanges between blood and tissue fluids remains unknown: transport by vesicles may account for all, or only for part, of these exchanges and only future quantitative work will tell us whether we are dealing here with the main mechanism of transport or with an accessory one of limited importance.

In any case it should be realized that transport across the endothelium represents only one step in the entire operation. There is a second barrier to be crossed, the basement membrane, and if in the experiments so far reported it did not markedly affect the passage of the tracer, this does not mean that it will behave in the same way under different conditions or in respect to other tracers. In fact there is good ground to assume that the basement membrane should play an important role in such exchanges, the principal reasons being the following: it is difficult to ascribe specificity to a transport in quanta and, moreover, there are types of capillaries in which the endothelium is discontinuous or fenestrated and in which the blood plasma gains direct access to the basement membrane, which appears to be the only continuous barrier in the wall of the vessels.

#### Tracer Experiments on Renal Glomerular Capillaries

Capillaries with a fenestrated endothelium are encountered in many viscera,<sup>8, 10</sup> and the fenestration of their endothelium becomes particularly extensive in the glomerular capillaries of the kidney. In addition to an extensively fenestrated endothelium, these capillaries are characterized by the existence of a third cellular layer—made up of the pseudopodia of the visceral epithelium. Their basement membrane is also thicker and apparently more substantial than in other capillaries, visceral or somatic. Glomerular capillaries represent a favorable object of study because in their case we are dealing with one-way transport only—i.e., from the lumina to the capsular space—and because the nature of the capsular fluid is relatively well known. It has been collected by direct micropuncture in a number of species and its analysis has shown that it is a protein-free, or almost free, filtrate of the plasma.<sup>cf. 17</sup>

In the experiments on glomerular capillaries, carried out in collaboration with Drs. M. Farquhar and S. Wissig, the tracers used were again ferritin and colloidal gold.<sup>18-20</sup> After short time intervals (3 to 15 minutes), the ferritin was found in high concentrations in the lumen. From the lumen it appeared to gain free access, through the fenestrae of the endothelium, to the basement membrane. Within the latter the tracer was found in noticeably lower concentrations and more or less evenly distributed in surface and in

Figure 9

*Renal glomerular capillary of a nephrotic rat one hour after the intravenous injection of 50 mg. ferritin.*

*The basement membrane (BM) crosses obliquely the field separating the epithelium (EP) at left from the endothelium (EN) at right. The lumen is hardly visible at the extreme right. Large deposits of ferritin infiltrate the spongy areas (sa) and the luminal layers of the basement membrane. Such deposits identify the basement membrane as the main filtration barrier. Fewer particles penetrate the peripheral layers of the filter and reach the epithelium where they can be seen in invaginations of the cell membrane (t<sub>1</sub>), in closed vesicles and small vacuoles within the cytoplasm (t<sub>2</sub>), and in dense bodies or absorption droplets (t<sub>3</sub>).*

*At this relatively late time point, the endothelium contains membrane-bound vacuoles (pb) filled with packed ferritin—an indication of the phagocytic activity by which the filtration deposits are removed. X 73,000.*



Figure 9 (See legend on opposite page)

depth. Few ferritin molecules reached the foot processes of the epithelial layer, and those that did appeared to be caught either in small invaginations of the cell membrane or in small vesicles within the cytoplasm. After longer time intervals (30 to 60 minutes), 2 new noteworthy features emerged: first, there was a gradual increase in the concentration of the marker in the luminal strata of the basement membrane and finally extensive piling up of ferritin molecules against its luminal surface; second, the number of ferritin molecules captured by the epithelium increased and, in addition to those located in membrane invaginations and small vesicles in the foot processes, the tracer appeared in the cell body proper in large vacuoles and in dense bodies.

To confirm these findings, we also administered the tracer to rats rendered nephrotic by treatment with the aminonucleoside of puromycin.<sup>21</sup> In such cases, the permeability of the glomerular capillaries is increased and substantial amounts of blood proteins are lost during glomerular filtration. In nephrotic animals, the piling of ferritin against the basement membrane was about as striking as in normals after long time intervals (fig. 9), but the amount of ferritin in the basement membrane and in the epithelium was considerably greater (fig. 10\*). The epithelium showed its typical response to the nephrotic condi-

tion: the extensive disappearance of the foot processes. In these altered epithelial cells, as in normal ones, the marker was restricted to membrane-limited spaces, i.e., vesicles, vacuoles, and dense bodies. In nephrotic animals there was a definite reduction in the extent of the fenestration of the endothelium.

From these experiments, it can be safely concluded that in this type of capillary the main filtration barrier is the basement membrane. The tracer is retained by it as by a filter. It is clear, however, that the filter is imperfect. Even under normal conditions, it leaks a detectable amount of the tracer, which is recovered, at least in part, from the filtrate by the epithelium that seems to function as a monitor of the filter proper. As expected, operations connected with this recovery are considerably enhanced when the filter becomes more leaky in nephrotic animals. Turning now to the filter proper, it should be pointed out that the ferritin molecules that escape through it were found distributed at random within the membrane. There was no preferred relationship to the slits of the epithelium, for instance. Moreover, and probably more important, no pores were seen in the basement membrane. The tracer molecules were found embedded in its substance without channels ahead or trails behind. Pores of simple geometry allowing the passage of a ferritin molecule would have a diameter in excess of 100 Å and should be visible. Since they are not, we are led to conclude that they are either extremely tortuous and consequently difficult to see in sections of the thickness used (~ 500

\*Figure 10 reproduced from Farquhar, M. G., and Palade, G. E.: Segregation of ferritin in glomerular absorption droplets. *J. Biophys. & Biochem. Cytol.* 7: 297, 1960. By permission of the Journal of Biophysical and Biochemical Cytology.

Figure 10

Part of a renal glomerulus in a nephrotic rat, 2 hours after the intravenous injection of 50 mg. ferritin.

Most of the field is occupied by part of an epithelial cell (EP), which contains the marker (ferritin molecules) in small vesicles ( $t_1$ ), small vacuoles ( $t_2$ ), large vacuoles with a light content ( $t_3$ ), structures of intermediary appearance ( $t_4$ ), and—finally—typical dense bodies ( $t_5$ ,  $t_6$ ,  $t_7$ ). It is assumed that all these forms represent progressive stages in the segregation and concentration of the marker and other materials incorporated by the cell from the glomerular filtrate.

The basement membrane (BM) of the capillary, covered by this epithelial cell, appears in the upper left corner infiltrated by numerous ferritin molecules.

The lumen is not visible in this field, but some urinary spaces can be seen (US).  $\times 73,000$ . (From Farquhar and Palade: *J. Biophys. & Biochem. Cytol.* 7:297, 1960.)



Figure 10 (See legend on opposite page)

to 800 Å), or that permanent pores do not exist. One has the impression that the marker moves through a yielding gel, creating a channel as it moves. As far as the activities of the other layers are concerned, they appear to be ancillary, if we restrict our interest to the filtration process proper and disregard morphogenetic processes for the moment. The endothelium seems to function as a valve that regulates the amount of plasma which gains direct access to the filter, whereas the epithelium behaves like a monitor which partly compensates for the imperfections of the filter. Analogies with the endothelium and the adventitial cells (primarily macrophages) of muscle capillaries are quite evident.

**Effects of Histamine and Serotonin upon the Structure of Small Blood Vessels**

In the light of these experiments on glomerular capillaries, should we conclude that the basement membrane is also the main filter in muscle capillaries, i.e., in capillaries with a continuous endothelium, and that in the latter the vesicles represent only a more refined "valve" than the fenestrae of the glomerular capillaries?

The last experiments to be reported suggest that such a conclusion is justified, at least in part. In collaboration with Dr. Guido Majno, and Miss Gutta Schoefl<sup>22, 23</sup> from the Department of Pathology at Harvard, we tried to find out what changes are introduced in the structure of blood capillaries by local histamine or serotonin treatment which is known to increase the permeability of small vessels markedly.<sup>24, 25</sup> The experimental device was simple and relied primarily on the discovery of a favorable specimen: the cremaster of the rat, a thin layer of striated muscle—2 muscle fibers thick—located under the skin of the scrotum. The marker, this time a coarser colloidal particle of mercuric sulfide (200 to 500 Å in diameter), was introduced into the general circulation; and histamine or serotonin, in doses of 50 µg. and 5 µg. respectively, was injected locally between the skin and the cremaster. The latter was excised and fixed at various time points thereafter either *in toto*, for light microscopy, or in small blocks,

for electron microscopy. The effect of the 2 amines becomes visible under the dissecting microscope in approximately 3 minutes, and spectacular in 10 to 15 minutes. It consists in a spotty blackening of the vessels that otherwise retain their usual appearance at low magnifications. The electron microscope provides a satisfactory explanation for the blackening.<sup>22</sup> The "lesion" produced by the amines is a local discontinuity in the endothelium, apparently caused by the pulling apart of 2 endothelial cells over longer or shorter distances. A gap is thus produced through which the plasma, loaded with the marker, gains direct access to the basement membrane. The latter apparently lets the water and many of the solutes pass, but as a filter retains the marker. In time, relatively large deposits of HgS particles accumulate within the wall of the vessel and start to dissect its layers. I should point out that the amines affect preferentially the small venules, as clearly indicated by the examination under the light microscope of cremasters mounted *in toto*,<sup>23</sup> but the lesions extend on the vascular tree toward the capillaries, at least toward their venous ends. In view of the preferential localization of the lesions, in itself a very intriguing finding, it is not surprising that the morphology of the affected vessels is sometimes extremely complex. Successive and unequal deposits are found in the thickness of the wall cleaving its various layers, which in the case of a venule can be more numerous than in the case of a capillary. In addition to the marker, many other circulating particles are retained by the filter. Frequently chylomicrons accumulate within the wall, together with deposits of proteins, some of which polymerize into tactoids with the periodicity of fibrin. Finally cellular elements, i.e., platelets, erythrocytes, and leukocytes, find their way into these dissecting aneurysms of the wall of the vessels. What is remarkable is the fact that, with all its tenuity and poor definition, the basement membrane of these vessels is capable of retaining the large deposits formed by the residues of filtration. Relatively few particles reach the pericapillary space and those that

do are rapidly picked up by phagocytic elements located along the vessels (fig. 8). The effect of histamine on the structure of blood capillaries has been surveyed by Alksne,<sup>26</sup> who arrived at the conclusion that the amine increased the pinocytic activity of the endothelium in addition to causing the formation of channels across the endothelial cells. The differences in results and interpretation between his and our experiments are due to differences in specimens and time points examined. In his case the specimen (skin) was less favorable and the timing inadequate. The results of the histamine and serotonin experiments indicate clearly that the basement membrane of the venules and capillaries of the cremaster behaves, when denuded, like that of glomerular capillaries: it proves its ability to function as a filter by accumulating a conspicuous filtration residue. Yet it is too early to conclude that the endothelium does not screen what it transports at all, for so far only a very small number of various kinds of particles have been tested.

#### General Comments

Admittedly much remains to be done before the relation between the structure and the function of various capillary vessels is clearly understood, but the findings so far recorded already suggest distinct roles for the successive layers of the capillary wall and point to the basement membrane as a functionally important component. This layer appears to be the best candidate for the role of selective filter. It remains to be seen, however, whether its selectivity can be explained by simple devices, such as pores of fixed geometry, or by more complex properties. In this respect the higher permeability of all capillaries to lipid-soluble substances (when molecules of similar diameter are considered) should not be forgotten. With this in mind, it is to be regretted that we know so little about the chemistry of the filter. Whatever we know is derived from histochemical tests which indicate that the basement membrane consists of a mucopolysaccharide,<sup>27</sup> probably conjugated or associated with proteins. More knowledge will

undoubtedly be helpful. I hope that the work presented may act as a stimulus in this direction.

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## Discussion

**Dr. Huxley:** I have a question about the structure of the reticulum in some muscles, where the triads are situated at the level of the Z-lines. Perhaps Dr. Fawcett could tell me how certain it is that the vesicular elements are continuous across the H-zone, from 1 Z-line to the next.

**Dr. Fawcett:** As I understand the question, it is: How do we know that the longitudinal elements of the reticulum are continuous across the H-zone? Is that right?

**Dr. Huxley:** Do you think that they are continuous?

**Dr. Fawcett:** There is no doubt in my mind that, in the fish muscle illustrated in my slides, the sarcotubules of the reticulum are continuous across the H-band. This is clearly seen in sections passing tangential to the broad face of the myofibrils. There is, however, considerable doubt about the continuity of the reticulum across the X-line.

**Dr. Huxley:** I asked this because Andrew Huxley, in his experiments with the micropipette, seemed to get the contraction localized very sharply in a longitudinal direction. Often the contraction did not appear to spread across the H-zone, so that only the 2 half sarcomeres on either side of the Z-line contracted.

**Dr. Fawcett:** I know of Dr. Andrew Huxley's experiments and cannot account for the localized response.

**Dr. Palade:** To explain Andrew Huxley's experiments, there should be some sort of a stop at the level of the H-band. There is no morphologic evidence for such a discontinuity at the level of the reticulum. Some years ago, however, Dr. Barnett and I demonstrated cholinesterase activity at the level of the H-bands along the thick filaments demonstrated this morning by Dr. Huxley. Maybe this enzyme is responsible for the stop in the spreading of the wave of contraction.

The physiologists would certainly like to see continuity, at some level in this system, from myofibril to myofibril, or, rather, from the reticulum level of 1 myofibril to the reticulum level of the next. Now, what is the situation with respect to this type of continuity?

**Dr. Fawcett:** There seems to be no doubt in the case of the toadfish muscle, which, geometrically, is more favorable for studying this point than other muscle, that there is continuity transversely across the entire face of any single myofibril, i.e., across the thickness of the contractile cylinder. The communication between the periphery of the contractile cylinder and the sarcolemma is more difficult to follow because of the meandering course that these tubules take in regions where they do not conform to the precise geometry of the myofibrils. But even in these regions, we can trace them sufficiently close to the sarcolemma to feel that, if we were able to obtain serial sections, we would find points of contact. There would then be continuity in a radial direction all the way from the sarcolemma to the core of the contractile cylinder. However, without an enormous number of serial sections, it would be almost impossible to prove that, in an ordinary skeletal muscle, there is continuity from the sarcolemma to the middle of the fiber.

**Chairman Kossman:** Dr. Fawcett, I should like to turn to another topic, namely, the difference between the concentration of mitochondria in cardiac muscle and in the very fast-acting skeletal muscles that you talked about. Do you have any physiologic explanation for this anatomic difference?

**Dr. Fawcett:** I have no very satisfactory explanation. I suppose that it is related to that fundamental difference between the skeletal and cardiac muscles, wherein the cardiac muscle has to keep up with its energy requirements currently, whereas the skeletal muscle

can incur some debt. Perhaps, the extraordinary number of mitochondria in cardiac muscle may improve its efficiency in meeting its energy requirements, which are continuous.

*Dr. Fishman:* I should like to direct a question to Dr. Palade. Although your presentation this morning indicated that the capillary endothelium may be continuous or discontinuous, it made no reference to pores in the basement membrane; these pores are generally held to be involved in the transport of fluids across the capillary walls. Quanta seemed to replace pores for this movement of fluids. But by what mechanism do gases move across myocardial capillaries.

*Dr. Palade:* There would be no problem there because, I suppose, gases move very easily across the cell membrane, and probably can move easily across the basement membrane.

*Dr. Fishman:* You believe then that water crosses the capillary wall by a different route from that traversed by gases?

*Dr. Palade:* What we are proposing is simply a discontinuous channel for a continuous channel. We are also pointing out that, in addition to discontinuous or continuous channels in the endothelium, diffusion or exchange across the basement membrane must still be taken into account.

It is easier to account for the movement of larger molecules by this mechanism than of water. As Dr. Fishman has intimated, this is a mechanism geared particularly to exchanges in which large particles or molecules are involved; other mechanisms involving pores have to be postulated for the mechanism of water. In any case, if the pores do exist, they are below the practical limits of resolution of the microscope, i.e., below 20 or 15 Å. The dimensions postulated have not been seen, and, again, they should have been seen.

*Dr. Fishman:* May I close this by asking: do you visualize any role for pinocytosis in this transport mechanism?

*Dr. Palade:* What I have described is somewhat comparable to pinocytosis. The only difference is that, instead of getting large and

more or less unequal particles picked up by the cell—i.e., droplets of fluid picked up by the cell—the vesicles are submicroscopic.

*Dr. Milton Landowne* (Baltimore, Maryland): Would you comment on the possibility that the sections are actually those of highly tortuous channels rather than vesicles?

*Dr. Palade:* This can be answered by studying serial sections, and serial sections show that the large majority of these vesicles are independent structures—that they are not part of a continuous system of tortuous channels.

*Dr. Rhodin:* In this connection, I should like to ask Dr. Palade if he thinks that the entire fluid transport occurs via these vesicles? Studies of the kidney have led us to believe that the intercellular space is utilized for the transport of fluid. Would this also apply to these capillaries?

*Dr. Palade:* I can only restate what I have already said. The markers that we used are not found at the level of the intercellular spaces. Some of these markers, i.e., the colloidal gold particles, are very small—of the order of 30 or 40 Å. Therefore, they would have access to pores, if pores of the order of 60 Å do exist at the level of the cell junctions or at the level of the so-called cement substance.

*Dr. Rhodin:* Dr. Huxley, I should like to ask if you have been able to see the fine cross-bridges between the myofilaments, which you demonstrated so beautifully here in preparations other than the glycerinated muscle? I am not questioning your results, but it would be pleasant to find them in ordinary fixed muscle.

The reason for bringing up this question is the knowledge of the results obtained by Sjöstrand and Andersson-Cedergren (*J. Ultrastructure Res.* 1:74, 1957) who suggested that each filament is composed, in turn, of subfilaments—unit filaments—arranged in a helical or, perhaps, in a coiled fashion.

*Dr. Huxley:* We can see the cross-bridges in preparations of frog sartorius muscle, for instance, or of one of the toe muscles. They do not show up so clearly as they do in the

glycerinated muscle, but they can be seen quite distinctly. As a matter of fact, I think they are visible in a picture of live rabbit sartorius muscle in a paper which I published in the *Journal of Biophysical and Biochemical Cytology*. You can see them quite clearly in insect flight muscle that has not been glycerinated.

**Dr. Rhodin:** Would you like to comment on the views of Sjöstrand? Do you think a coiled structure does not exist in these filaments?

**Dr. Huxley:** Well, I think that the cross-bridges we have seen seem to project out from the myosin filaments at regular intervals along the length of the filaments and point toward each of the 6 surrounding actin filaments in turn, as though they were located on a helical course. This pattern, as well as the tapered appearance of the filaments at their ends, would fit in very nicely with the idea that the bridges were made up of smaller subunits, myosin molecules, with from 10 to 20 myosin molecules lying side by side within the diameter of each filament, and many in series along its length.

**Dr. Weidmann:** Dr. Rhodin, did you really expect conduction velocity to increase with an increasing number of intercalated discs per unit length, other things being equal?

**Dr. Rhodin:** Yes.

**Dr. Weidmann:** I cannot quite follow the argument. If you increase the number of discs per unit length, the electric resistance inside the fibers can only increase. And, according to cable theory, the propagation velocity then drops. Even if you assume something like a functional transmission from 1 "cell" to the next, I have difficulty in seeing how a higher number of such sites should speed up conduction. Propagation across more synapses can only be associated with more delay.

**Chairman Kossmann:** Dr. Rhodin, do you wish to comment on this?

**Dr. Rhodin:** Not at the moment.

**Dr. Hoffman:** I have 2 questions. In the capillary, the basement membrane seems to be a diffusion barrier. I wonder if the outer layer of the sarcolemma, which is not continuous with the sarcoplasmic reticulum, as far

as I know, might be analogous to the basement membrane. It could thus be our desired diffusion barrier, whereas the inert layer of the sarcolemma, which is continuous with the intracellular structures, might perhaps be a structure of very high permeability? Is there any reason to consider the muscle membrane this way?

**Dr. Fawcett:** I will simply say that the muscle fibers in cardiac muscle do have an extraneous coating that, in all respects, resembles the basement membrane of the capillaries.

**Dr. Palade:** This outer layer is similar to the structure of the basement membrane and to the periphery of every muscle fiber in the skeletal musculature, as well as in the myocardium. It is different from the plasma membrane. It can be removed from the plasma membrane. It may definitely act as a tissue barrier for large particles, as in the case of the blood capillary.

**Dr. Podolsky:** I should like to ask Dr. Huxley how he managed to get the 2 sets of thin filaments to slide past each other. Couldn't they have coiled first and then slipped past, after the contraction was over?

**Dr. Huxley:** That preparation was glycerinated muscle that was shortening in ATP under load. After it had shortened down the required amount, the ATP was washed out of it, so that instead of going into the relaxed state after the contraction was over, it went into a state of rigor.

Now, on any of the models, even ones depending on a coiling of the actin filaments where you envisage an interaction of the cross-bridges with the actin filaments, you would think that, when a glycerinated muscle came to the end of its shortening and the ATP was washed out, the bridges would become attached permanently to the actin filaments instead of repetitively; this attachment would hold the actin filaments in the position they occupied while the shortening was going on. Then there would be no opportunity for them to uncoil again—to straighten out—in the center of the sarcomere, as Dr. Podolsky suggests.

I agree that this type of observation on a

living muscle during contraction would be more difficult to interpret. But, in this case, the effect you see may be significant.

*Dr. DeHaan:* Dr. Rhodin, it has been pointed out that one of the differences between myocardium and the specialized system that Dr. Rhodin has just presented is the lack of a basement membrane in the conduction system. Have you seen this difference?

*Dr. Rhodin:* There is a difference. In the myocardium, as well as in the S-A and A-V nodes, each cell is surrounded by a basement membrane. This membrane is continuous at the site where the cells adjoin, that is, at the

intercalated disc. However, in the common bundle and its branches, the entire set of cells in each tiny bundle—3 to 4 cells in width—usually is surrounded by the basement membrane; so the functional units in the fiber are all surrounded by basement membranes.

*Dr. Hoffman:* Dr. Rhodin, where were your sections of the A-V node from?

*Dr. Rhodin:* The ones that I described as being A-V nodal fibers were taken from the approximate middle of the node. Of course, as you approach the common bundle, there is a direct continuity between the nodal fibers and the common bundle fibers.

### Myocardial Metabolism—1924

When a muscle contracts, tension is developed, and external work is done if the tension is made use of to raise a weight or perform other functions requiring expenditure of energy. It is obvious, therefore, that there must be something in resting muscle which possesses potential energy of some kind, and that, on excitation, some change takes place in this system resulting in loss of potential energy. We know that lactic acid is formed and that the actual contractile process is not associated with the giving off of carbon dioxide nor with the consumption of oxygen. It is not, in fact, an oxidation, so that the "biogen" conception fails here. Although there must be some large molecules, or aggregates, containing the lactic acid group, these cannot be of a protein nature with "intramolecular" oxygen as one side chain and an oxidisable group at another place. At the end of the contraction, the cell machinery possesses less potential energy and the systems actually participating in the change, "inogens," if we use Hermann's name, though not exactly in his sense, have let loose lactic acid.

Now to restore the system to its original state, with increase of energy content, a further, exothermic, reaction is necessary. In this process the system is restored to its original state of high potential energy, so that the reaction by which it is effected must be one in which a considerable amount of energy is set free. This is shown by the large consumption of oxygen and liberation of carbon dioxide, indicating oxidation of some combustible substance. We have seen already . . . that no nitrogen metabolism is associated with muscular work as such; the oxidised substance must therefore be carbohydrate or fat. It appears that carbohydrate is normally used in the muscle itself, but fat appears also to be capable of serving the purpose, perhaps indirectly, in the intact animal.—W. M. Bayliss. *Principles of General Physiology*, Ed. 4. London, Longmans, Green & Co., 1924.

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## II. Biochemistry

Chairman: John V. Taggart, M.D.

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### Introduction

By JOHN V. TAGGART, M.D.

IN THIS morning's session, a great deal was said about ultrastructure in the myocardium, notably concerning the myofilaments, the endoplasmic reticulum, and the mitochondria, as well as the capillaries. We now know that many of these structures possess specific biochemical and enzymatic properties.

This afternoon's program will deal with selected metabolic features of the myocardium. In an effort to gain greater insight into the relationship between structure and function, we shall be concerned mainly with the so-called mechanochemical transformation

that underlies muscle contraction, that is, the conversion of chemical energy into mechanical work. As has been pointed out by others, the study of muscle contraction follows many paths and involves a variety of experimental techniques. One of these is the analysis of structure, about which we have already heard. Another is the characterization of the component proteins of these structures. The third deals with the enzymatic activities of the proteins and the fourth with a consideration of theoretical model systems. Finally, we shall return to the physiology and metabolic regulation of the intact tissue.

Because one of the reactions most frequently implicated in the process of muscular contraction is the hydrolysis of ATP, it is appropriate that we begin on this note.

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### Biological Thinking and Investigation

Yet I feel so sure of two broad principles which should govern all biological thinking and investigation that I should be lacking in courage and honesty were I to neglect to bring them before you. The first is that there is no limit to the degree to which the mechanisms of life can be explored and elucidated by what are commonly called physical and chemical means: nature has erected no barrier beyond which "trespassers will be prosecuted," and arranged no desert into which it is profitable to go. The second is that however far we get we shall still find "function," "adaptation," "organisation," "purpose," in the processes we explore: the purpose may seem queer sometimes, the function may be unexpected: the adaptations may prove imperfect, but viewed as a whole they will be found to represent the best compromise available.—A. V. Hill. *Living Machinery*. New York, Harcourt, Brace & Co., 1927, pp. 277, 278.

## The ATPases of Muscle Proteins

By MANUEL F. MORALES, Ph.D., AND SHIZUO WATANABE, Sc.D.

The ideas and results of Blum, Oosawa, Strohman, and many others are reviewed and, when consolidated with the authors' work, lead to the following picture of muscle protein adenosine triphosphatases (ATPases). Two major features of myocin ATPase seem to be a catalytic interaction between enzyme, a metal cation, and the terminal pyrophosphate moiety of adenosine triphosphate (ATP) and a rate-retarding interaction between enzyme,  $Mg^{++}$ , and the purine ring of ATP. Sulfhydryl groups of the enzyme participate at both loci. In the catalytic interaction an ionizable group ( $pK$ , ca. 6.8) may participate. G-actin molecules binding ATP (probably by the purine ring) and relieved of their mutual repulsion cooperate in catalyzing dephosphorylation, thereby giving rise to the somewhat loose, possibly helical, structure of F-actin.

**I**N CONTRIBUTING to this symposium we are tacitly assuming that, as regards our subject, there is no substantial difference between heart and skeletal muscle tissue. It has also to be said that, although ATPases are emphasized in the title and in the paper itself, it is very possible, in view of work such as Strohman's,<sup>1</sup> that *in situ* one or more of these enzymes function in phosphate transfer rather than in ATP hydrolysis. Such a possibility would not vitiate the merit of ATPase studies, since the objectives in most of these studies are clues about enzyme surfaces and enzyme-substrate interactions. For these purposes it is quite legitimate to consider various "acceptors," among them water. At the same time, we must acknowledge that, even if these studies were totally successful and we understood precisely the mechanisms of the enzymes, we would still have to integrate our information with that of other speakers in order to understand how muscle "works."

In this paper we shall consider primarily the ATPase activities of myosin and actin, but first it is convenient to touch upon some properties of ATP and its relatives.

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### Substrates

For the enzymes to be considered, the "natural" substrate appears to be adenosine triphosphate (ATP). A long-known property of ATP is that its free energy of hydrolysis is substantial. A possible consequence of this fact for interactions of ATP with its enzymes is the plausibility of formation of energetically costly enzyme intermediates. Other conceivable properties of ATP as a substrate, such as charge and binding affinities of its various moieties, have generally received scant attention. In recent years, however, interest in ATP-metal cation complexes has been kindled by mounting evidence that metal cations play a critical role in ATPase and contractile properties.

It is safe to assume that metal cations are chelated by the polyphosphate end of ATP and may form a sort of electrostatic "cement" linking substrate to enzyme and facilitating distal hydrolytic attack by water molecules. However, enzymologic investigations have prompted speculation that metal cations also interact with the ring end of ATP, either to anchor it to enzyme or to its own polyphosphate end. The existence of these interactions can be and has been investigated in the absence of enzyme. To establish further that these interactions are part of the "normal" enzymatic process—for example, to show that the "true substrate" is a metal complex—is much harder; it has not been done decisively

for either myosin or actin, although most recently it has been argued for the related enzyme, creatine kinase.<sup>2</sup>

The thermodynamic affinity of metal cations for ATP (without regard for the structure of the complex) has been studied by several competent investigators and methods, and it is therefore disturbing that the results have varied widely. The hypothesis that the adenine ring participates in metal binding was first put forth by Blum,<sup>3</sup> who sought to account for a ring-enzyme anchor point, and shortly thereafter by Levedahl,<sup>4</sup> Szent-Györgyi,<sup>5</sup> and Eichhorn,<sup>6</sup> all of whom noted that simultaneous chelation of metal ions by ring and polyphosphate moieties would stabilize ATP in a curled configuration of possible functional significance. Although Levedahl's measurements<sup>4, 7</sup> can blend with such a hypothesis, direct evidence for ring-metal cation interactions has been wanting. Recent preliminary observations<sup>8</sup> by K. Hotta and J. Brahms in our laboratory may help resolve the difficulties clouding this problem. Hotta and Brahms found that if the affinity (formulated for a 1 nucleotide: 1 metal cation complex) is measured at various concentrations of metal cation, there is an inverse correlation between affinity and concentration. This trend is also noticeable in the calculations of other authors who employed methods of varying sensitivity and therefore worked at different concentrations. The implication is that not only a 1:1 complex but also a 1:2 complex exists. Hotta and Brahms have not yet completed their study, but for purposes of orientation we shall mention that at an ionic strength  $[(\text{CH}_3)_4 \text{NCl}]$  of 0.05 M and total nucleotide and  $\text{MgCl}_2$  concentrations of  $10^{-3}$  M and  $10^{-2}$  M respectively,  $K_{298}$  (ATP) =  $8 \times 10^3$ , and for inosine triphosphate (ITP)  $K_{298}$  (ITP) =  $12 \times 10^3$ , where "K" is the apparent 1:1 affinity in li/mole. These values are from pH meter measurements in the neighborhood of pH 6.8, i.e., they reflect displacement by cations of protons attached to the polyphosphate structure. Protons are also displaceable from the rings of ATP, ITP, and cytidine triphosphate (CTP) at charac-

teristic pH's. In these cases, proton detachment (whether by reaction with  $\text{OH}^-$  or by cation displacement) is accompanied by measurable shifts in the ultraviolet spectra of the nucleotides, and from these shifts one may infer the affinities of the cations for the ring moieties of the various nucleotides.\* Taking advantage of this effect, Hotta and Brahms have found that, for the rings,  $K_{298}$  (ATP) =  $1.6 \times 10^2$  li/mole and  $K_{298}$  (ITP) =  $1.5 \times 10^2$  li/mole.† They have also found appreciable ring-binding with the diphosphates, ADP and IDP, but not with monophosphates, ribosides, or free bases. Taken together, these various observations are strong support for the general assumption that metal cations can interact with the ring atoms of nucleotides; they also indicate that a (small) fraction of the nucleotide-metal cation complexes are in the curled configuration. However, it remains for other experiments to decide whether these configurations have a functional significance (for instance, for fitting into enzyme crevices).

In principle, both gross and subtle properties of different portions of the ATP structure can be studied by observing the behavior of analogs (fig. 1). Thus, it has been established in a gross way that orthophosphate is hydrolyzed only from a triphosphate structure (e.g., ATP or tripolyphosphate, PPP, not ADP or pyrophosphate, PP). In view of PPP, the attachment of a purine or a pyrimidine ring and ribose to the triphosphate structure is inessential for myosin triphosphatase activity, but it modifies such activity. Since ribose triphosphate is not yet available, one cannot decide whether the enhancement stems

\*It is logically possible, though much less likely, that the metal cation bound to the polyphosphate structure is not simultaneously "touching" the ring, but only attenuating the electrostatic field that otherwise perturbs the ring protons.

†Although the ring affinities for ATP and ITP are about the same, it should be recalled that around neutral pH (e.g., 8) the proton competition for the ATP-ring site will be nil, whereas the proton competition for the ITP-ring site will still be formidable; accordingly, the fraction of ring sites holding cation will be much greater in ATP than in ITP.

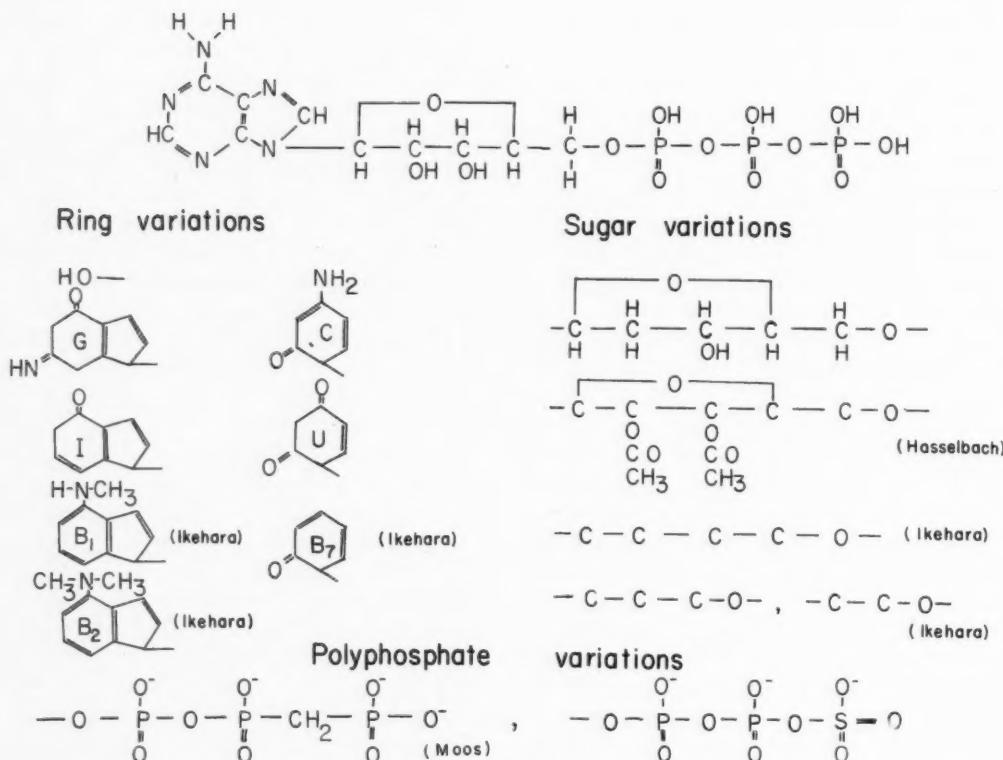


Figure 1

Some substrates for ATPase.

from the ring, from the ribose moiety, or simply from the elimination of an extra negative charge in PPP. From the analogs of Hasselbach<sup>9</sup> and Ikehara et al.,<sup>10</sup> it appears that considerable liberties can be taken with the ribose region without suppressing myosin activity; in fact, it may be that reducing the enzyme-ribose interaction enhances activity. On the other hand, the fact that either a purine or a pyrimidine moiety permits active catalysis suggests that specific and simultaneous interaction between several ring atoms and enzyme is not obligatory or that there is flexibility in the enzyme structure. Another type of interesting analog, adenyl-methylene-diphosphonate (adenyl-PCP), was recently introduced by Moos et al.<sup>11</sup> Even though identical with ATP in every steric respect, adenyl-PCP is peculiarly inert toward the muscle

proteins. In this molecule, however, "opposing resonance" is suppressed and probably causes the pK for the final phosphate ionization to shift from 6.5 to 8.2 and reduces  $Mg^{++}$  chelation at neutral pH. We are thus again reminded of the importance of the nucleotide charge for substrate capability.

With the advent of diverse analogs such as those introduced by Ikehara et al. and Moos et al., a number of other deductions will undoubtedly be forthcoming. An increasingly definite deduction from analogs already available will be discussed in the next section.

#### Myosin

Elsewhere in this symposium, Olson, and Bing and Kato, discuss the physical properties of myosin, but it is cogent for us to know now that to date the smallest kinetic unit reported for solutions of purified *skeletal* myosin

has a mass of not less than  $4 \times 10^5$  Gm. Various attempts—the latest being that of Nanninga and Mommaerts<sup>12</sup>—to measure the myosin weight that combines with 1 mole of ATP or of PP have led to very nearly this same value. Therefore, although we are unconvinced\* we have to agree that on present evidence the ATPase site of myosin seems to occupy but a tiny portion of the molecule. Speculation about the function of this tiny site has been dominated recently by two ideas that we shall now consider.

#### The Double Displacement Scheme

By analogy with certain other hydrolases there has been proposed (see, for instance, Weber<sup>13</sup>) for myosin a so-called "double-displacement" reaction scheme:



Justification of such a scheme has been attempted along 3 lines: (1) According to the scheme, a  $P^{32}$  label introduced in the form of ADP should back-incorporate into ATP. Although early European reports seemed to bear out this expectation it now seems very definite that the back incorporation is not observed with pure muscle proteins. (2) Under appropriate conditions of isolation, it ought to be possible to interrupt the reaction and to demonstrate enzyme with co-valently attached phosphorus. This has also been reported, but such experiments are equivocal owing to possible sorption of inorganic phosphorus by the active enzyme. Maruyama and Gergely,<sup>14</sup> for instance, have recently concluded that this sorption, rather than any

true phosphorylated intermediate, explains this sort of experiment. (3) It is possible that the myosin-catalyzed excess incorporation of  $O^{18}$  from  $H_2O^{18}$  into inorganic phosphorus (produced from ATP) indicates a phosphorylated intermediate, since it does not occur in either ATP or inorganic phosphorus. However, Koshland and his associates,<sup>15</sup> who discovered the  $O^{18}$  exchange, have shown since that its rate is sensitive to purine-ring substituents, so that for all practical purposes the exchange occurs while the ATP (or other nucleotide) is still intact, and thus its existence is not evidence for a phosphorylated enzyme. In summary, experiments that with other enzymes have eventually demonstrated an intermediate have, in the case of myosin, been quite inconclusive thus far.

Before attempting to discuss a second major hypothesis about the myosin-ATPase mechanism, it is convenient to consider the general classes of substances that modify this ATPase.

#### Substances That Modify Myosin ATPase

##### Salts in General

In his pioneering work Szent-Györgyi<sup>16</sup> showed that the chlorides of various metals had a di-phasic effect on both the solubility and the ATPase activity of myosin. At neutral pH, low concentrations of salts precipitated the enzyme and activated ATPase, while greater concentrations solubilized the enzyme and inhibited the ATPase. Szent-Györgyi felt that the solubility phenomena resulted from cation binding (which rendered the protein isoelectric) followed by anion binding (which charged the protein negatively), and he considered that, although diverse cations catalyzed ATPase, the precise effect of a particular concentration depended not only on the specific cation but also on the ionic strength. Unfortunately, these early discoveries and ideas have not been widely examined or pursued. As regards ATPase, there has arisen a complicated literature in which investigators report the pH, the cation used, and the ionic strength. KCl has been regarded as an inert electrolyte that provides only ionic strength, and the possibility that anions might have

\*Reasons for doubt are both subjective and objective. Other enzymes, once really purified, have invariably proved to be of a smaller order of magnitude, so it is puzzling to be faced with such an exception. A more scientific doubt arises from the recent recognition that the binding of ATP to enzyme can be strongly inhibited by polyanions. Investigators of site weight have borne in mind the possibility that the true substrate is an Mg-ATP or Mg-PP complex,<sup>12</sup> so to reduce complications the system has generally been saturated with Mg salts, sometimes with  $MgSO_4$ . Not only may the increased ionic strength have discouraged binding to enzyme, but also  $SO_4^{2-}$  may have competed successfully for erstwhile ATP sites.

specific effects has been largely disregarded; thus a report may read, "Mg<sup>++</sup> (actually MgCl<sub>2</sub> or perhaps MgSO<sub>4</sub>) is an ATPase inhibitor at ionic strength of 0.60 M (actually in 0.60 M KCl)." As a result of preliminary work with solvents more nearly "inert" than KCl, we now know that such statements are treacherous, since superimposed on the truly ionic strength effects of these electrolytes is a competition between K<sup>+</sup> and Mg<sup>++</sup> (and no doubt H<sup>+</sup>) for specific binding sites and also specific Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> binding, which may be competitive with ATP binding.<sup>2</sup> Nevertheless, the statements that follow are tentative because our work has not been extended to a sufficient number of ions. As regards solubility, it appears<sup>17</sup> that the precipitation when electrolyte is first added is predominantly a nonspecific ionic strength—presumably screening of solubilizing repulsion—effect. The subsequent solubilization, however, is as Szent-Györgyi surmised the result of anion binding, and the higher the anionic valence the greater the solubilization per mole of anion. As already anticipated by Mihalyi,<sup>18</sup> Cl<sup>-</sup> is certainly bound; such anions as sulfate, ferri-cyanide, and pyrophosphate are even more strongly bound. As in the creatine kinase system, acetate seems fairly "inert."<sup>22</sup> Among the cations we have reason to think that tetramethylammonium is also "inert" (see below). Our best current approximation to an inert electrolyte is therefore tetramethylammonium acetate; however, because of its ready availability, we have also used tetramethylammonium chloride (TMAC), especially for low ionic strengths. At an ionic strength of a few tenths TMAC, myosin ATPase is soluble but essentially inactive. If now a metal chloride is added at increasing concentration (but still low enough so as not to change the ionic strength markedly) the ATPase activity rises to a maximum level and stays there. As would be expected from all past work, CaCl<sub>2</sub> gives the highest asymptotic activity of the simple salts thus far tested; the activity provided by KCl, however, is appreciable. Used in this way, MgCl<sub>2</sub> is definitely an activator, although much weaker than CaCl<sub>2</sub>. The particular ac-

tivity attained with a salt is undoubtedly the resultant of co-catalytic effects, such as those mentioned in the substrates section, and inhibitory effects such as will be proposed later for Mg<sup>++</sup>. Provisionally, however, we will assume that Ca<sup>++</sup> is solely an activator—possibly just functioning in P-O-P hydrolysis; thus in many experiments the solvent will be a solution of an inert electrolyte and an enzyme-saturating concentration of a suitable calcium salt. Of course work of other laboratories, to be cited below, will meet these conditions only roughly.

#### *Specific Sulfhydryl Reagents*

In their classical work on SH enzymes, Singer and Barron<sup>19</sup> early showed that myosin could be totally inhibited by parachlormercuribenzoate (PCMB), so it has been long presumed that the enzymatic site of myosin contains, among others, a sulfhydryl group. More recently, Kielley and Bradley<sup>20</sup> showed that, besides this group at the site, there existed in the molecule a second, more reactive type of sulfhydryl group (probably not at the site), the masking of which accelerated the Ca<sup>++</sup>-saturated ATPase activity at neutral pH. On this account a plot of activity vs. number of SH groups titrated is diphasic; as the more reactive groups are affected the activity is increased, then as the less reactive groups react the activity falls (eventually to zero). Blum<sup>21</sup> has shown that other heavy metals besides mercury behave in the same way. The purely "activation" response is best evoked by the class of reagents that form mixed disulfides with myosin when incubated at moderately high pH, e.g., by S-β-aminoethylisothiuronium (AET). Dinitrophenol behaves qualitatively like PCMB and AET, and, although it has never been clearly shown to be an SH-reagent, it probably belongs with this class of ATPase modifiers. Kielley and Bradley<sup>20</sup> speculated that the most PCMB-reactive SH groups in myosin might in some way chelate the inhibitory "intrinsic" Mg<sup>++</sup> (see below). This speculation has been supported by the subsequent observation<sup>22</sup> that AET-treated myosin ATPase is less sensitive to Mg<sup>++</sup> in-

hibition, and very recently by Tonomura's observation<sup>23</sup> that "intrinsic"  $Mg^{++}$  (not removable from myosin by ethylene diaminetetraacetate [EDTA] rinsing) is released by the enzyme on reaction with PCMB.

*Substances That Chelate Alkaline Earth Cations*

Through the work of Tarver-Friess<sup>24, 25</sup> and of Bowen<sup>26</sup> it was established that at neutral pH EDTA strongly accelerated the ATPase of myosin B dissolved in 0.60 M KCl and inhibited that of myosin B dissolved in 0.60 M KCl. The result was no different if the enzyme was first rinsed with EDTA and then with KCl solution before adding the final test EDTA. Recrystallized KCl was used throughout. Since in the test system  $Mg^{++}$  (and only  $Mg^{++}$ ) might have been expected to inhibit at 0.60 M KCl and activate at 0.60 M KCl, it was speculated<sup>25</sup> that some form of inhibitory  $Mg^{++}$  bound to the enzyme so tightly as to defy EDTA rinsing was being masked by the test EDTA in the activation phenomenon.\* It is this  $Mg^{++}$  that Kielley and Bradley<sup>20</sup> thought might be held by SH groups on the myosin.

*Hydrogen Ions, or pH*

Although the dependence of myosin ATPase activity upon pH has been known for two decades, its uninterpretable shape (at face value the curve shows optima around pH 6.4 and pH 9.5) has led to no useful inferences. The attainment of high ionic strengths with minimal specific ion effects (by the use of TMAC), however, has enabled some progress. It has been shown<sup>28, 29</sup> that for AET-treated myosin<sup>†</sup> the  $Ca^{++}$ -saturated ATPase activity curve is like a titration curve with a "pK" between 6.5 and 7.0, and not very dependent

on 1/T (very similar characteristics to creatine kinase,<sup>2</sup> and to the pH dependence of  $Zn^{++}$  inhibition of contraction of glycerinated muscle fiber<sup>30</sup>). The curve for untreated myosin ITPase has very much the same shape. On the other hand, as pointed out by Gilmour,<sup>31</sup> the curve for untreated myosin ATPase (in which pains have been taken to avoid irreversible effects at high pH) looks like an ITPase curve in which some form of inhibition has been imposed at pH 7-8. It is cogent to recall here that Bowen's curves of EDTA-activated myosin ATPase as a function of pH<sup>26</sup> also had a sigmoid (rather than bi-optimal) aspect—rather like titration curves spread out over many pH units.

**Single Displacement Followed by Desorption**

We now return to discuss what we have called the second major hypothesis about the myosin-ATPase mechanism; this is a hypothesis developed especially by Blum, but with notable independent contributions by Koshland, Levy, and their associates, and by Gilmour. These authors suppose that, while all substrates interact with myosin at the terminal pyrophosphate grouping, those substrates (e.g., ATP, CTP) with an  $NH_2$  group suitably placed on either the purine or pyrimidine ring suffer a strong additional interaction with the SH-bound "intrinsic"  $Mg$  of the enzyme. According to Blum, this ring-enzyme interaction retards the desorption of the diphosphate following P-O-P hydrolysis, thus effecting an inhibition of what would be the activity in the absence of interaction. Gilmour<sup>31</sup> has additionally assumed that this retarding interaction is pH sensitive and maximal at pH 7-8. To account for certain temperature effects, Koshland and Gilmour separately have proposed that at some temperature between 20 and 0 C. a conformational change in the enzyme draws away the SH-Mg site from the substrate so that below the transition temperature the retarding interaction is no longer possible. In contrast to ring- $NH_2$  substrates, substrates such as ITP are assumed to be free of retarding interaction.

Like all tentative hypotheses, the foregoing

\*This speculation is being disputed in current work from Tonomura's laboratory,<sup>23</sup> since these workers find that after dislodging its intrinsic metals with PCMB the enzyme still responds to EDTA. However, a basic difficulty may lie in the use of KCl contaminated with Mg salts; we have found<sup>27</sup> that in TMAC solution EDTA does not exert its characteristic effects. For the while we feel the Mg hypothesis continues to have merit.

†Similar results were obtained contemporaneously by J. Blum, using  $Cu^{++}$ -treated myosin ATPase.

leaves many facts unexplained (why is ITPase inhibited by small degrees of SH titration? Why would an NH<sub>2</sub>, Mg, SH interaction respond to pH change around 7-8?), but it has many attractive consequences. For example, the hypothesis readily explains the pH curves, why reagents as different as AET and EDTA cause similar effects, and why Mg<sup>++</sup> is not an inhibitor of ITPase activity. Excluding mathematical booby traps (see Morales and Hotta<sup>29</sup> for discussion), the foregoing observations on pH dependence also point to an ionizable group at the active site, with a pK of 6.5-7.0—possibly an imidazone or a phosphate of the enzyme-substrate complex.

Recently, we performed experiments<sup>27</sup> whose results are encouragingly consistent with the general Blum-Koshland-Gilmour hypothesis. We measured ATPase and ITPase activities as a function of pH, in both ordinary and heavy water. On the assumption that the isotope effect manifests itself primarily on the hydrolytic (P-O-P splitting) process, we asked at each pH, does D-for-H substitution make a difference in the observed rate? In the case of ITP, the substitution always made a difference, i.e., the rate in D<sub>2</sub>O was always less than the rate in H<sub>2</sub>O. In the case of ATP the substitution made a difference at the pH extremes, but at pH 7 or 8 it made no difference at all. From our premise we concluded that the ITPase rate is probably always limited by the hydrolytic process and that this is also true of the ATPase rate at the pH extremes, but that around pH 7-8 some other process is limiting the ATPase rate. This "other process" would be the inhibitory ring-enzyme interaction; if so, it should be eliminable by AET- or by DNP-treatment. This prediction was confirmed; with these treated enzymes the substitution did affect the ATPase rate, even at pH 7-8. What the final fate of this ATPase theory will be depends on future research, but its present status seems bright.

#### Actin

Among the important properties of actin is its transformation from individual "monomer" units (M.W., ca.  $6 \times 10^4$  Gm.) to

more or less endless polymers, i.e., from its "G" to its "F" form, upon such changes as the addition of salts or the reduction of the pH. Straub, and Laki and Bowen, discovered independently that in the G-F transformation the ATP tightly bound to G-actin was dephosphorylated in the course of the transformation. Later, Mommaerts showed a precise 1-to-1 correspondence between the moles of orthophosphate released and the moles of G-actin brought into polymer form. The matter stood at this stage for several years, except that during the interim Laki repeatedly emphasized the curious parallelism between conditions that govern myosin ATPase and those that govern actin polymerization, and he raised the question of whether actin was an ATPase. At the 1957 Tokyo Symposium on Muscle Chemistry this question was affirmatively answered by Oosawa, Asakura, and their associates at Nagoya University. These workers showed that under suitable conditions (see below) an actin system could catalyze the *continuous* dephosphorylation of ATP, not merely the "one shot" amount that accompanied the conversion of almost pure G to almost pure F. Before discussing actin ATPase, mention must be made of another, seemingly contradictory, discovery by Oosawa's laboratory. According to these workers, the polymerization of actin is cooperative, i.e., if one commences with a very dilute solution of G-actin and raises the G-actin concentration, a "critical" G-actin concentration (dependent on [Mg<sup>++</sup>], pH, temperature, etc.) is reached beyond which the further addition of G-actin results in the progressive appearance of F-actin whilst the concentration of G-actin stays at its critical value. Such behavior is typical of systems in which several of the condensing monomer units are *simultaneously* interacting in the formation of polymer. According to the Nagoya workers an actin system exhibits maximal ATPase activity when G and F actins are co-existing, both in appreciable amounts (condition G-F). For a given value of total actin concentration, the ratio G:F will therefore vary with the state ([Mg<sup>++</sup>], temperature, etc.), and, for a given state,

the ratio will vary with the total actin concentration. The claim is that whatever are the state and total concentration that achieve G-F, that combination also maximizes the ATPase rate. This elegant result is also a powerful indication that the ATPase is not arising from contaminations, as was once seriously proposed. Yet we have always felt that a fundamental difficulty might exist, since, if polymerization and dephosphorylation are rigidly linked, then dephosphorylation as well as polymerization would be cooperative. This circumstance would be quite unprecedented. Each ATP being dephosphorylated by a myosin molecule, for example, is very remote and insensitive to the fate of fellow ATP molecules being dephosphorylated elsewhere; thus far, interacting enzymatic sites have existed only in the minds of theorists.<sup>32</sup> It therefore seems to us very important that in their latest paper Asakura, Kasai, and Oosawa<sup>33</sup> provide evidence that actin ATPase is cooperative. For example, they find that upon the addition of salt the initial rate of splitting depends on a high power (3.5) of the G-actin concentration, and that the addition of F-actin causes a burst of ATP splitting. To provide a structural basis for the polymerization and ATPase effects, Oosawa is now proposing a helical (rather than linear, to account for interactive effects), possibly salt-linked, and rather delicate structure for F-actin. In support of this structure he has already shown acceleration of ATP splitting by 10 Kc. sonic irradiation. Apart from being contributions to the knowledge about ATPase, the foregoing observations are opening the way for a possible "intrinsic contractility" in F-actin, a matter of critical concern to the model of contraction discussed in this symposium by Dr. Podolsky.

Although the "enzymology" of actin is still too sketchy to discuss at length, mention should be made in closing of Strohman's<sup>1</sup> important new observations on actin-ATP binding. The absorption of  $Mg^{++}$  and  $Ca^{++}$  to actin monomers undoubtedly reduces repulsions and facilitates polymerization; very possibly it also facilitates hydrolytic attack on P-O-P. However, Strohman now has evidence

that such cations cooperate with SH groups of actin in the highly specific (ITP won't do) binding of ATP to the protein. His observations are very reminiscent of the "retarding interaction" of ATP discussed above for myosin.

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## Muscle Physiology and Contraction Theories

By RICHARD J. PODOLSKY, PH.D.

The structural basis of current contraction theories is the double array of thick and thin myofilaments revealed by the electron microscope. The physiologic properties that characterize the contractile process are natural consequences of this structure if (a) during shortening the 2 sets of filaments move relative to each other and (b) the flux of chemical energy through the contractile mechanism is limited by interaction between complementary sites distributed along the 2 sets of filaments. Contraction models fitted to these ideas differ largely in the mechanism by which force is generated. In the *sliding model*, force is developed by mechanical interaction between the thick and thin filaments, and filament length remains constant during contraction. In the *folding model*, force is developed in the thin filament, which shortens during contraction. Both models quantitatively account for the force-velocity relation and the Fenn effect. They also accommodate the quick-release phenomenon and predict, at least qualitatively, the isotonic velocity transients that can be seen after quick release from tetanic tension.

**B**IOCHEMICAL PROCESSES in muscle cells have 2 exceptional characteristics. The first, of course, is that they generate large forces. The second, which is not quite so obvious, is that they proceed at a rate which depends on the motion of the cell. Many properties of contracting muscle can be traced back to the influence of the motion of the cell on the chemical processes driving the contractile mechanism, an idea first proposed by Fenn.<sup>1</sup>

It seems very natural that a muscle should lift a light load more rapidly than a heavier load. However, as Fenn wrote some years ago, "the more one tries to explain these simple facts, the less obvious do they seem to be."<sup>2</sup>

The "simple" fact is shown in figure 1, the force-velocity curve for the classical striated muscle, the frog sartorius. The circles are data from an experiment we shall discuss later. The smooth curve is A. V. Hill's force-velocity relation.<sup>3</sup> The question is: Why does the contractile force fall with the velocity?

In the early twenties, the force-velocity relation was explained with a viscoelastic model<sup>4</sup> along the following lines: Upon activation, muscle becomes an elastic filament, like a spring. The spring is immersed in a viscous fluid. When the muscle shortens a given distance, the available potential energy

appears either as work or as heat. The more quickly the muscle moves, the greater the viscous force, the greater the heat production, and the smaller will be the energy available for work. Thus the fall in force with speed.

The essential part of the viscoelastic model is that, in shortening a given distance, the available energy is independent of the mechanical conditions of contraction. The corollary, that the amount of heat produced in a contraction increases with the speed, was doubted as long ago as 1864.<sup>5</sup> The unambiguous experiments of A. V. Hill confirmed these doubts.

Figure 2, taken from Hill's classical 1938 paper,<sup>3</sup> shows the critical experiment.\* Curve E is the total heat produced as a function of time when the frog sartorius is tetanized isometrically. Curves F, G, H, and J show the heat liberated when the muscle is released, at the time indicated by the first arrow, and allowed to shorten a given distance. Although the speed of contraction in J, say, is 5 times greater than in F, the total extra heat due to shortening does not change. Since it was clear that heat production did *not* increase with speed, the viscoelastic theory was demolished.

\*Figure 2 reproduced from Hill: Proc. Roy. Soc. London s.B 126: 136, 1938.<sup>3</sup> By permission of the Royal Society of London.

From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland.

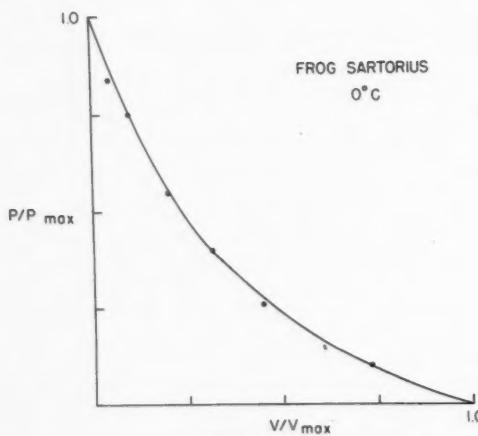


Figure 1

Relation between force,  $P$ , and velocity,  $V$ , in living muscle. Experimental points are calculated from data of figure 12; smooth curve is the force-velocity equation of A. V. Hill.<sup>3</sup>

The error, of course, is that the energy available during contraction is not constant. The flux of chemical energy into the muscle is somehow affected by the motion itself. In other words, some kind of mechanochemical control is built into the contractile mechanism. The force-velocity relation might be a reflection of this mechanochemical control if the tension generating process were continuously opposed, or inactivated, by the mechanical motion.

This idea is schematized in figure 3. We assume that a sequence of chemical reactions drives the contractile mechanism. One of these reactions, **M**, is closely linked to the contractile mechanism. Since the chemical reactions are themselves linked like a train of gears, the extent of the reaction during a contraction is limited by the turnover of **M**, which, in turn, is some function of the shortening velocity. The total energy produced during contraction depends on the extent of the reaction **A** + **M**. This chemical energy\*

\*In this paper, "energy" denotes the enthalpy of reaction,  $\Delta H = \Delta U + P\Delta V$ . The energy available for mechanical work is the free energy,  $\Delta H - T\Delta S$ . In these expressions,  $U$  is internal energy,  $P$  is pressure,  $V$  is volume,  $T$  is temperature, and  $S$  is entropy.

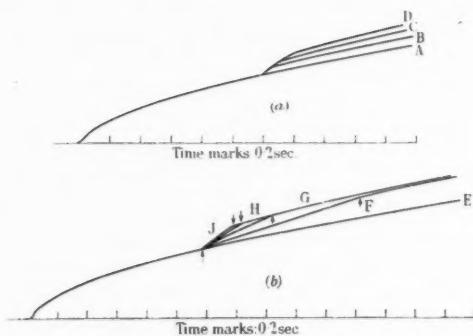


Figure 2

Heat production of living muscle. Tetanically stimulated frog sartorius at 0°C. (a) **A**: isometric contraction. **B**, **C**, **D**: 1.2 sec. after start of stimulus, muscle is released and allowed to shorten various distances (**B** < **C** < **D**) under constant load. (b) **E**: isometric contraction. **F**, **G**, **H**, **J**: muscle is allowed to shorten the same distance under various loads (**F** > **G** > **H** > **J**). (From Hill.<sup>3</sup>)

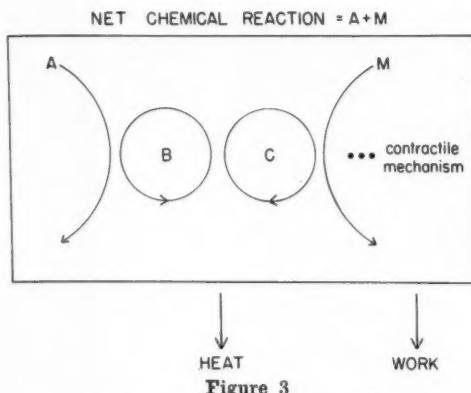
is partitioned between work and heat. Conversely, the rate of chemical reaction can be inferred from the rate of total energy production. The trick in devising contraction theories is to make the link between mechanical and chemical processes such that both the total energy production and its partition into work and heat depend on mechanical motion in just the right way. We shall first show what the "right way" is, and then describe how several models manage to do this.

Before leaving figure 3, I should like to mention that there is independent evidence, from certain heat measurements, that this representation of the sequence of events is close to the truth.<sup>6, 7</sup> Perhaps the best justification, though, is that it accommodates the physiology of muscle very comfortably.

#### Energy Production

How does the rate of chemical reaction change with the rate of mechanical contraction in living muscle? This can be inferred from the velocity dependence of the energy flux (fig. 4).

Consider the heat first. You will recall that Hill's heat measurements showed that the heat of shortening is independent of contraction speed (fig. 2). This means that



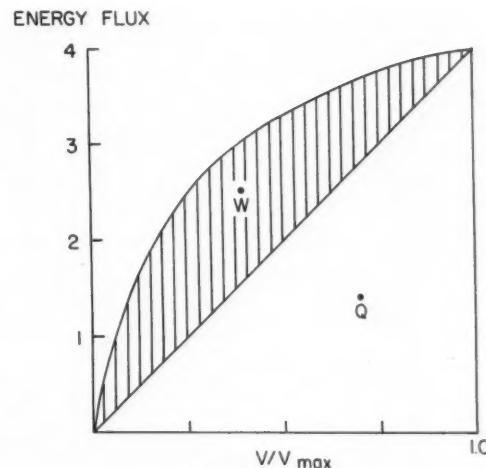
Scheme for chemical processes associated with muscular contraction.

the rate of heat production must increase linearly with speed (fig. 4, open region).

The rate of work production also depends on speed: the dependence can be calculated from the force-velocity relation. When the muscle does not move ( $V = 0$ ) it produces no work. Also, when it is unloaded ( $P = 0$ ) no work is done. This ties down the ends of the work-flux curve (fig. 4, shaded region). Adding the heat to the work, we see that the total energy flux increases monotonically, but not linearly, with speed. The rate of the rate-limiting chemical reaction, the one linked to the contractile mechanism, must also increase with speed in exactly the same way. To be acceptable, a contraction theory should yield this relation naturally and quantitatively.

#### Structural Basis of Contraction Theories

What mechanism regulates muscle chemistry according to speed? The double array of filaments revealed in Hugh Huxley's beautiful electron micrographs<sup>8</sup> provides a clue. Figure 5 shows the characteristic thick and thin filaments.\* The thick filaments define the A-band. A second set of thinner filaments extend from the Z-line, through the I-band, into the A-band, there interdigitating with



Relation between energy flux and velocity in living muscle. Open region: rate of heat production. Shaded region: rate of work production.

the thick filaments. The part of the structure that interests us is diagrammed at the top of figure 6.

Andrew Huxley and Rolf Neidergerke demonstrated that when living muscle shortens, the decrease in length takes place almost entirely in the light I-bands, the width of the dark A-bands remaining constant.<sup>9</sup> There are two obvious ways for this to come about: the filaments could slide along each other or, after anchoring its ends, the thin filament could shorten by folding (fig. 6). In both schemes only the I-band shortens. Also, and this is important in what follows, in both schemes there is *relative motion* between the 2 sets of filaments. This means that if reactive sites were distributed along both the thick and the thin filaments, and if some kind of interaction between these sites were stoichiometrically linked to the driving chemical reaction, the relative motion of the myofilaments—and therefore the sites—would provide a natural basis for introducing *velocity* as a parameter in the chemical kinetics.

#### Implications of Relative Motion: Energy Production

The basic idea is shown in figure 7. Sites

\*Figure 5 reproduced from H. E. Huxley: J. Biophys. & Biochem. Cytol. 3: 631, 1957.<sup>8</sup> By permission of the Journal of Biophysical and Biochemical Cytology.

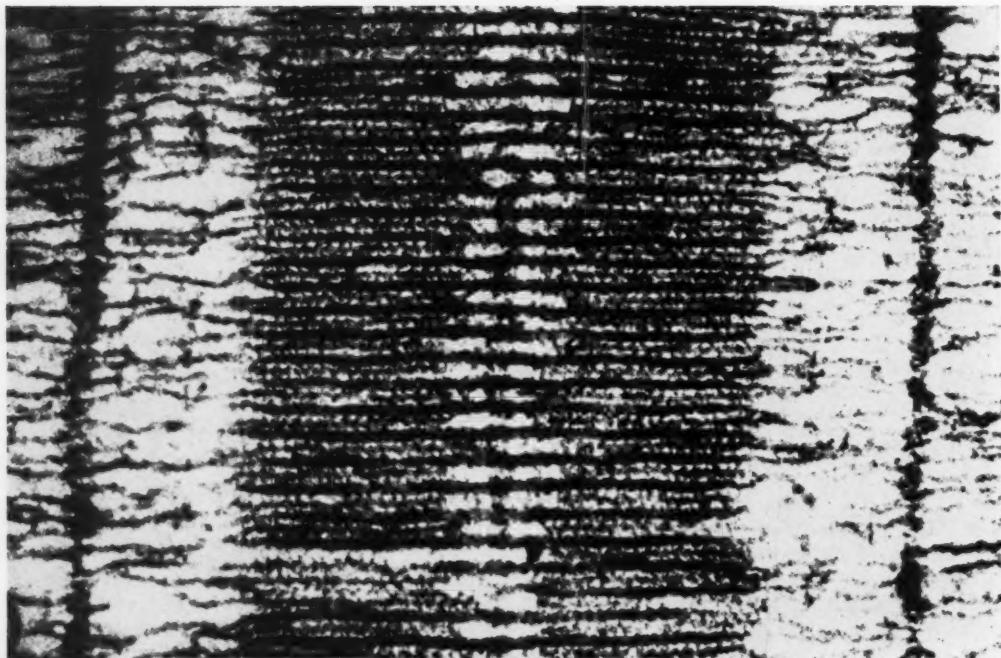


Figure 5

Double array of filaments in striated muscle. Electron micrograph of longitudinal section of saromere (length = 2.5 microns). (From H. E. Huxley.<sup>8</sup>)

D are distributed along the thick filament and complementary sites, K, are distributed along the thin filament. We assume that interaction *may* take place when these sites pass each other. If interaction does take place, a substrate molecule, M, is used up, and the driving chemical reaction proceeds one step.\*

#### Proximity Time Is Rate Limiting

To understand the influence of velocity in the kinetics of such systems, consider first a case in which the probability of interaction depends on the time K spends in the neighborhood of D: the shorter the time, that is, the higher the speed, the lower the probability will be. The interrupted line in figure 8 shows how the probability decreases with speed if the K-D interaction is first order in

time. Since at each interaction the driving chemical reaction proceeds 1 step, the interaction probability in a transit of K past D will also be proportional to the energy released for a *given amount* of shortening. The decrease in this quantity with velocity can be interpreted as the Fenn effect.<sup>1</sup>

To calculate the number of interactions per unit of time, we must remember that the number of chances a given K site will have to interact with D sites—the encounter rate—is proportional to the velocity (dotted line, fig. 8). The number of *successful* interactions will be the product of the probability and the encounter rate (solid line, fig. 8). Both this function and the rate of the driving chemical reaction in living muscle, calculated from the energetics (fig. 4), depend on velocity in the same way. We conclude, then, that if complementary discrete sites were distributed along the 2 kinds of myofilaments, the rela-

\*In the following, a helpful mnemonic is to read D as "Dragon," K as "Knight," and M as "Maiden."

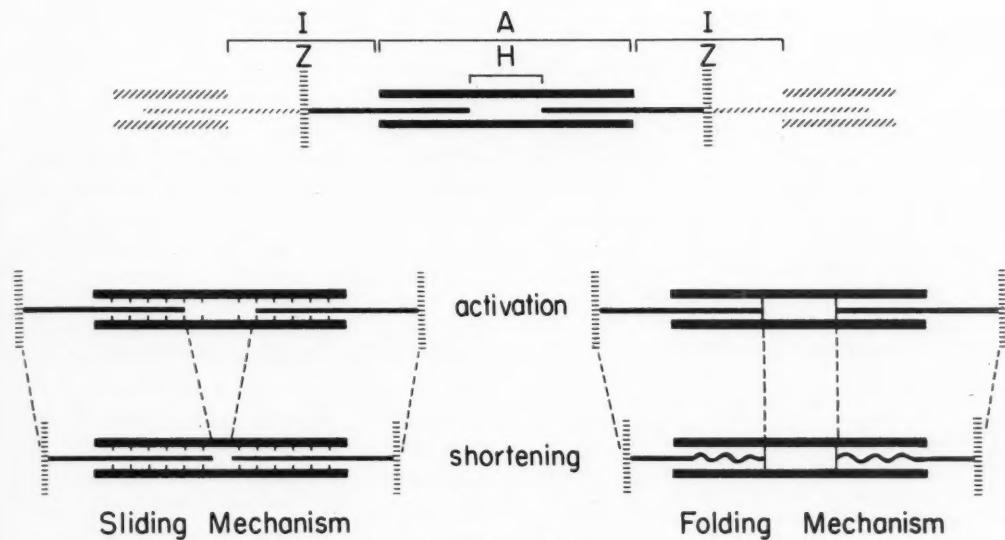


Figure 6

*Hypothetical mechanisms for muscular contraction. Upper: Configuration of thick and thin filaments in resting muscle (after fig. 5). Lower: Change in filament shape and configuration in sliding and folding contraction models.*

tive velocity of the myofilaments could control the rate of interaction between these sites in exactly the same way as it controls the release of chemical energy in living muscle.

#### Filling Time Is Rate Limiting

A physically different way of describing interaction between sites on moving filaments focuses on the time spent *between*, rather than *at*, encounters. In this case we suppose that the substrate molecule, M, is carried by K past D. If K is loaded with M, an interaction takes place at D, emptying K, *regardless* of the speed. The rate-limiting step in this scheme is the filling of K with another M after it has been emptied by D. The filling probability will be lower the shorter the time spent between D sites, that is, the higher the speed. It turns out that if the law for filling empty sites is exponential in time, which is not unreasonable, the probability factor will have exactly the same form as in the previous case, in which "proximity time" rather than "filling time" is rate limiting.<sup>10</sup> This means that there will be no difference in the kinetics

of the 2 schemes for steady motions; both explain equally well how motion controls the rate of energy release. The different structure of the schemes will become important, however, when we consider velocity and force transients.

The question of whether the thin filaments fold or slide during shortening has been sidestepped. This could be done because in *steady* motions the mathematics proves to be substantially the same for both cases.<sup>7</sup>

#### Force

What generates the contractile force? In a sliding model, since the lengths of both the

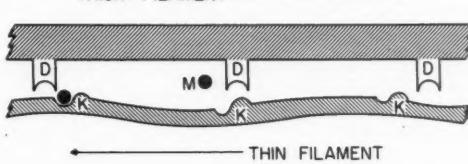


Figure 7

*Distribution of sites along the thick and thin filaments. See text for explanation.*

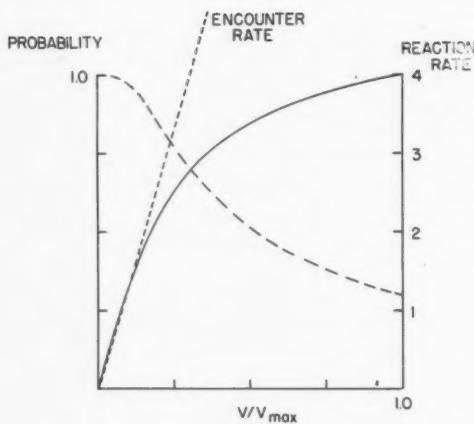


Figure 8

*Chemical kinetics for reaction sites moving with relative velocity. V. Interrupted line: probability of interaction (rate limited by proximity time) or of filling (rate limited by filling time). Dotted line: encounter rate. Solid line: reaction rate = probability  $\times$  encounter rate.*

thick and thin filaments remain the same, the contractile force must stem from mechanical interaction between the filaments. In a folding model, however, the contractile force is generated in the thin filament and its length is supposed to decrease during shortening.

#### A Sliding Model

The mechanical and chemical properties of a sliding mechanism in which the D site on the thick filament has the mechanochemical properties diagrammed in figure 9 was worked out by Andrew Huxley.<sup>11\*</sup> D oscillates back and forth. When the K site passes, it can interact with D to form a mechanical connection. Since the probability of forming a connection in a D-K transit depends on the relative speed, this is a special case of interaction in which "proximity time" is rate limiting. Each connection is, in time, broken. One step of the driving chemical reaction is associated with each connect-disconnect cycle.

If D and K connect, the elastic elements holding D to the thick filament will exert a

force on the thin filament that is proportional to the distance of D from O. If the thin filament is moving to the left, connections with D to the right of O will make a positive contribution to the force. Conversely, if motion of the thin filament carries D to the left of O, a force will be developed that retards the motion.

To develop a net positive force—that is, to ensure that there will be a greater number of pulling than retarding connections—Huxley postulates that D can connect to K only when it is to the right of O. The rate constant for breaking a connection also depends on x: it is small when D is to the right of O but large when it is to the left.

The force developed by the model depends on speed because the number of attachments and their distribution about the equilibrium position of the D site depends on speed. This is shown in figure 10, taken from Huxley's paper.<sup>11</sup> The ordinate is the fraction of D sites at a given displacement from the equilibrium position that are connected to K sites. When the filaments do not move, all the connections are on the pulling side of the equilibrium position: links can be made only on this side and there is no motion to carry them to the other side. In steady motion, the number of pulling links decreases; the retarding links tend to increase, and the force drops. At the maximum speed, the pulling and retarding forces balance and there is no net force. Huxley showed that, as the relative speed increases, these shifts in both number and distribution of links between filaments can account quantitatively for the force-velocity relation in living muscle.

The diagram also shows why there *must* be steady motion for a less-than-maximum force to remain constant. Consider the distribution of connections when the speed say, is one-tenth of the maximum. If the motion should stop, after some time pulling links would form to the right of O, retarding links would open to the left of O, and the net force would increase. The original force could be re-established by relaxing the pulling links

\*Figures 9 and 10 reproduced from A. F. Huxley: *Progr. Biophysics* 7: 255, 1957.<sup>11</sup> By permission of Pergamon Press Inc.

or stretching the retarding links, that is, by displacing the "connection contour" to the left, which, of course, is what happens when the thin filament slides past the thick filament. A steady force can be set up only when the motion in a given time interval just compensates for the net increase in pulling links formed in that same period.

The diagram also explains what happens if tetanized muscle is quickly released. Before release, the links are all in the pulling position, as shown in the distribution at the top of the figure. If the muscle is moved so quickly that the links do not change their points of attachment, the tension will fall linearly as the links are carried past the equilibrium point and will vanish when the distribution becomes symmetrical about the origin. Thus the model predicts that, if muscle is moved very quickly, the tension will drop to zero with a small displacement, which is what actually happens.<sup>12, 13</sup> This is a simple mechanical process and is due to the release of strain in the relatively short pulling connections between the thick and thin filaments.

Notice that the distribution of links just after a quick displacement will be rectangular, while the corners are rounded in the steady state. Since, for a given tension, the motion depends on the shape of the "connection contour," this implies that the velocity just after a sudden drop in tension from the maximum to some intermediate value will not be the same as that after the steady state has been established. There should be a velocity transient reflecting the transition of "connection contour" from a rectangle to the steady-state shape. We shall return to this point later.

In summary, in the contraction model analyzed by Huxley, the 2 sets of myofilaments slide past each other. The sliding motion arises from mechanical interactions between complementary sites. The chemistry of the system reflects the mechanical motion, since each mechanical interaction is coupled to a chemical reaction. The model explains remarkably well the structural and energetic

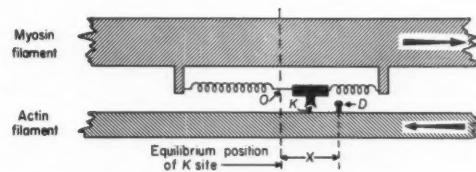


Figure 9

*Mechanochemical element in sliding model of A. F. Huxley. x is distance of thin (actin) filament site, K, from equilibrium position of thick (myosin) filament site, D. See text for further explanation. (From A. F. Huxley.<sup>11</sup>)*

changes that take place during contraction. The least satisfactory element in it is the rather special nature of the hypothetical mechanochemical sidepieces on the thick filaments, the "pullers." However, "it is not difficult for Nature to do things in ways which seem unduly complicated to physiologists."<sup>12</sup>

#### A Folding Model

In a folding model the contractile force arises from a change in state of the thin filaments; the thin filaments become elastic, like a rubber band. To make such a model work, we must assume that force is generated when a substrate molecule, M, binds to (or reacts with) the thin filament at a K site and that the magnitude of the force is proportional to the number of occupied K sites.<sup>14</sup> The force could arise from an "electrostatic entropic" process, as has been eloquently argued by Morales and Botts,<sup>15</sup> or from a "polymer melting" process, as suggested by Pryor<sup>16</sup> and by Flory.<sup>17</sup>

In an "electrostatic entropic" process, a rubber-like filament is stretched out by the mutual repulsion of distributed, electrically charged groups. At the equilibrium length, the entropic force tending to shorten the filament is just balanced by the electrostatic force tending to extend it. If some of the charged groups were neutralized by the binding of oppositely charged molecules, the net electrostatic force would decrease and the filament would tend to shorten; if length were kept constant, force would be developed.

In a "polymer melting" process, the filament is initially extended by structural forces.

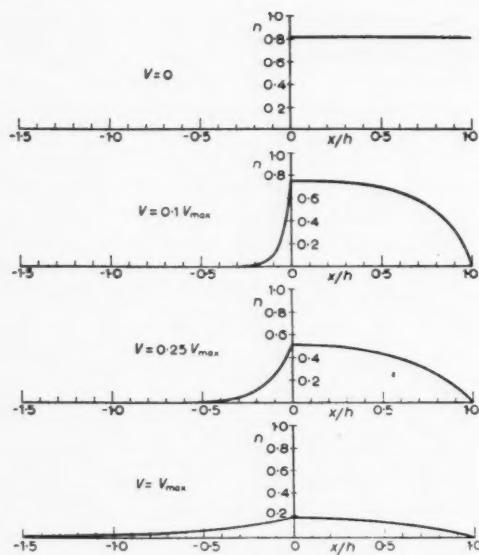


Figure 10

*Distribution of links between thick and thin filaments in steady motion of sliding model of A. F. Huxley: n is fraction of D sites at a distance x from the equilibrium position that are connected to K sites; V is relative velocity; h is maximum value of x (see fig. 9). (From A. F. Huxley.<sup>11</sup>)*

such as those that coordinate a crystalline solid. When a critical temperature is reached, the crystalline structure "melts" and the polymer becomes like rubber. In this state, if kept isometric, the filament will exert considerable force. The dotted line in fig. 11 shows such a phase transition for an ideal crystalline polymer. The melting temperature will be changed if substances bind to (or react with) the polymer; the change will depend on the extent of the binding.

Sharp phase transitions are characteristic of the ideal crystalline polymer. Melting curves of real polymers are often more gradual (solid line, fig. 11);<sup>17</sup> however, in this case too the curve can be shifted by an amount that depends on the extent of binding. Now, if temperature is kept constant ( $T = \tau$ ) and the extent of binding varied, the extent of melting will vary. If length is kept constant, the force will increase with binding.

Degree of melting in this process is analo-

gous to charge neutralization in the electrostatic-entropic process; both unlock the rubber-like properties of the polymer. The essential features are that (a) force is generated in a single filament which can shorten by folding and (b) force can change according to the number of small molecules bound to (or reacted with) the filament.

A considerable amount of muscle physiology follows naturally from this hypothesis if we suppose, further, that there are sites on the thick filament that remove the force generating molecules from the thin filament by interacting chemically with them as they move by. In other words, we suppose that there are interactions between the 2 sets of filaments in which "filling time" is rate limiting. We have already shown that this scheme yields up the correct answers for the relation between rate of energy production and speed.\*

To explain how the force-velocity curve comes out, consider figure 7 again. Suppose the force is a third of the maximum. Then 1 binding (K) site out of 3 will be filled; 2 out of 3 will be empty. A substrate molecule from the environment will, in time, find its way to one of the empty sites. When the site fills, the force in the filament will rise above that of the load. To re-establish mechanical equilibrium, the filament will shorten by folding until 1 of the filled sites passes a D site, which removes an M so that the force will again be a third of the maximum. The process will be repeated when another binding site is filled. In the steady state, with many sites in the game, the rate of motion will be such that the emptying of full K sites passing D sites is just balanced by filling of empty K sites. The force is the average occupancy of the K

\*The energetics of steady motion does not depend on whether "filling time" or "proximity time" is rate limiting. However, in the latter case (as was used in an earlier version of the folding model<sup>14</sup>), it can be shown that the model does not accommodate the drop in force after quick release from tetanic tension. On the other hand, if "filling time" is rate limiting, the folding model behaves "properly" upon quick release.

sites; the dependence of the average occupancy on velocity is the force-velocity relation. This turns out to be the same as that in living muscle.

What happens when a muscle exerting maximum tension is suddenly released? (You will recall that in the sliding model, because the "pullers" relaxed, the tension dropped linearly with shortening.) In this case, if the motion is quick relative to the filling time, full K sites will be rapidly emptied by reacting chemically with passing D sites, and the tension will drop. The tension will fall linearly with distance only until the force reaches half maximum. Then, because shortening is by folding rather than by sliding, it can be shown that further motion will lead to a proportionately smaller drop in force, which is what happens in living muscle.<sup>13</sup>

As in the analogous situation in the sliding model, just after a quick drop in force, the distribution of filled sites along the filament is not the same as it will be somewhat later, after the steady state is established. This means that the isotonic velocity after quick release from tetanic tension will generally be different from the later steady velocity. Some time must pass before the velocity settles down to the characteristic steady value.

#### Mechanical Transients

We made a series of experiments to look for this transition phase.<sup>18</sup> The study was made with tetanically stimulated frog sartorius at 0°C. (fig. 12).\* The upper trace is *displacement* of one end of the muscle and the lower trace is *force* at the other end. After full tension was developed, the force on the muscle was suddenly lowered to, and then maintained at, a given value. Each set of traces is for a different final value of the force. The insert (fig. 12g) is a control with the muscle replaced by a simple spring.

Three regions are of interest. To the left of the vertical line we see the quick release phenomenon. The muscle is 35 mm. long; the tension drops to nearly zero when the end

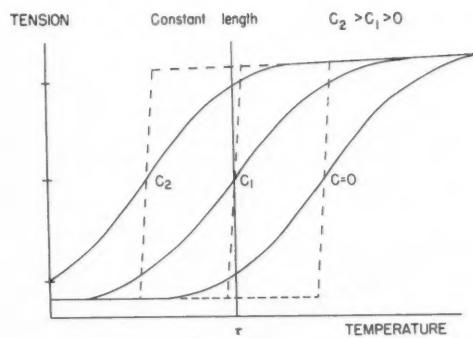


Figure 11

*Tension development according to "polymer-melting" process. Interrupted lines: melting curves for ideal crystalline polymers; solid lines: melting curves for real polymers. The melting temperature decreases with the extent of binding (or reaction) of substrate with the polymer filament.*

moves less than 2 per cent of this length (fig. 12f). Depending on which model we favor, this could be either relaxation of pulling links or emptying of binding sites. Hill found a burst of heat during the quick release, which he attributed to a high thermoelastic coefficient of what in the sliding model corresponds to the pulling spring.<sup>19</sup> An alternative interpretation is that a chemical rather than a physical process is associated with the loss of tension, as is the case in the folding model. This interpretation also agrees with the studies of the insect physiologists, who invoke "inactivation by release"—as opposed to "relaxation by release"—to explain the very high frequency movements of certain insect muscles, such as those driving the noisemaker of the locust.<sup>20</sup>

At each force, the velocity ultimately settles down to the characteristic steady value: the lower the force, the more rapid the motion. (The force-velocity curve in figure 1 was drawn from these data.) The remarkable linearity of the displacement traces supports the idea that the motion is controlled by a feedback mechanism.

The interesting findings in these experiments are the variations in speed (to the right of the vertical line) before the velocity settles down to the steady value; there is a

\*Figure 12 reproduced from Podolsky: *Nature* 188: 666, 1960.<sup>18</sup> By permission of *Nature*.

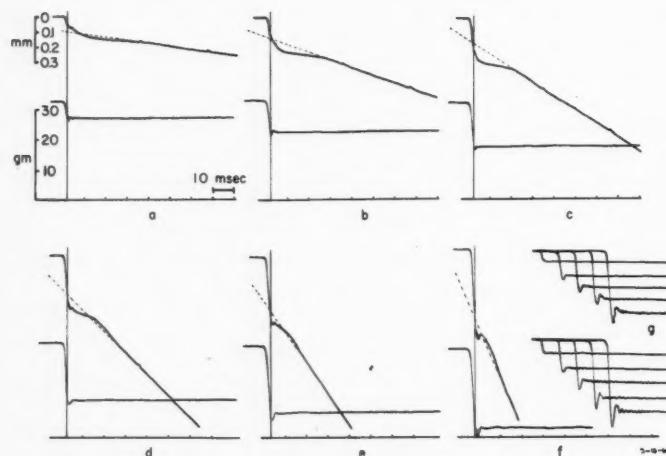


Figure 12

Response of muscle to a sudden change in force. Upper trace: displacement; lower trace: tension; frog sartorius, standard length = 35 mm., 0 C. Muscle is initially at the standard length and the record is started after full tetanic force,  $P_{max}$ , has developed.  $P/P_{max}$ : (a) 0.84 (b) 0.69 (c) 0.55 (d) 0.40 (e) 0.26 (f) 0.10. In (g) the muscle is replaced by a simple spring. (From Podolsky,<sup>18</sup>)

characteristic *isotonic* velocity transient for each tension step. One contribution to these transients is the variation in velocity corresponding to the setting up of the steady state of motion in the contraction models we have discussed. However, since the experiments were made with the whole sartorius muscle, there is also a contribution due to the interaction of muscle fibers of different lengths. To sort out these 2 components of the transient seen in the intact muscle, we are repeating these experiments with preparations containing only a few fibers; this should reduce the contribution of fiber interaction. We are also calculating the transients predicted by the sliding and folding contraction models, to see which model accommodates the experimental data better.\* In these studies attention is focused on the *approach* to the steady state of motion rather than on the steady state *per se*; then the contraction kinetics of the 2 models can be distinguished.

#### Conclusion

In summing up, I should like to point out that the contraction theories work because of 2 basic assumptions. The first—and this can really be elevated to the status of fact rather than assumption—is that the 2 sets of myo-

filaments move relative to each other in shortening. The second is that the flux of chemical energy through the contractile mechanism is limited by interactions between complementary sites distributed along the 2 sets of filaments.

Two models were fitted to these ideas. They differ largely in the mechanism by which force is generated. In the *sliding* model, force is developed by mechanical interaction between filaments and there is no change in filament length during shortening. In the *folding* model, force is developed in a single filament, which shortens during contraction. In both models there is interaction between the mechanical motion and the force-generating mechanism. Chemical processes tend to increase the mechanical force: in the sliding model, pulling connections are made; in the folding model, binding sites, which generate force if filled, become filled. If the load is constant, these processes tend to create a mechanical imbalance which, however, can be righted by shortening: in the sliding model, pulling connections become weaker; in the folding model, binding sites are emptied. Chemical and mechanical equilibrium are incompatible for forces less than full tension. However, since shortening tends to inactivate the force generator, a less-than-maximum force can be maintained if there is steady motion. Conversely, if the load is less than

\*This study is being made in collaboration with Dr. N. Z. Shapiro of the National Institutes of Health.

the maximum force that can be generated, there *will* be steady shortening. This is the force-velocity relation.

Comparing the models with living muscle, both of them quantitatively account for the changes in force and energy flux with velocity. The quick-release phenomenon is also accommodated: mechanically in the sliding model and chemically in the folding model. Both predict, at least qualitatively, the isotonnic velocity transients that can be seen after quick release from tetanic tension.

This list of accomplishments suggests that some of the devices used to get them might actually be built into living muscle.

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## The Regulation of Metabolism and Energy Release in Contracting Muscle

By WILFRIED F. H. M. MOMMAERTS, PH.D.

A brief review has been given of our present knowledge of the nature of the chemical events coupled with muscular contraction. Although there are experiments that fail to demonstrate a breakdown of ATP in the contraction of living muscle, these may yet yield to special explanations, leaving the conclusion that a breakdown of ATP is the reaction most closely related to the mechanical activity of the contractile structure. Owing to a rapid rephosphorylation reaction, this event appears in the form of a breakdown of phosphocreatine. The amount of energy liberated in contraction may be written as the sum of 3 quantities (a) activation energy, (b) shortening energy, and (c) work. It appears that, chemically, these correspond to 3 distinct quantities of phosphocreatine breakdown. The matter is complicated by the fact that the activation energy is not constant but may diminish as the muscle shortens. This may be little noticeable in frog sartorius muscle (dependent on the treatment) but appears to be pronounced in the heart, where it leads to the classical observation that work against a high pressure is performed less efficiently than work raised by ejections of an increased volume.

**I**N MORE THAN one respect, this opportunity to discuss the biochemical origin of muscle energy, and the mechanism of the regulation of its release, comes at an inconveniently early moment. I would have preferred to postpone it until after the completion of some of our current lines of experimental work. However, there is no certainty that this work will be successful within the foreseeable future; and it may be of interest to the audience to discuss the present status of the question and to present our preliminary views and the remaining formidable problems, as we now see them.

Most of our knowledge about the problem at issue has been obtained through the myothermal studies of A. V. Hill, and it may be well to give a brief outline of these first. The significance of such measurements is that they give a complete accounting of the total energy that is mobilized, as far as this is not turned into external work; the latter can be measured mechanically—or also thermally if at the end of the contraction the work is returned to the muscle (i.e., if the lifted load

falls back while stretching the muscle) and so warms it up by an amount equal to the work done originally.\* Furthermore, the myothermal method also allows us to establish the time course of energy liberation to a degree with which biochemists may eventually hope to catch up, but certainly have not done so yet. Thus, it has been established that, in a strictly isometric contraction, a definite amount of energy is liberated during the contraction phase of the cycle; this energy is called the activation heat  $A$ , or, alternatively, the maintenance heat, especially in the case of a tetanus when one emphasizes the maintenance rather than the initial establishment of the active state.

\*That the work done upon a relaxing muscle appears quantitatively as heat is not as self-evident as it might seem. One expects this when work is dissipated by friction, as in one of the Joule experiments, but not, e.g., when the lead is hung on a steel wire which cools when so stretched reversibly, because of the preponderance of the energy term  $\frac{\delta U}{\delta L}$  in the equation:

$$\left( \frac{\delta W}{\delta L} \right)_T = \left( \frac{\delta U}{\delta L} \right)_T + T \cdot \left( \frac{\delta S}{\delta L} \right)_T.$$

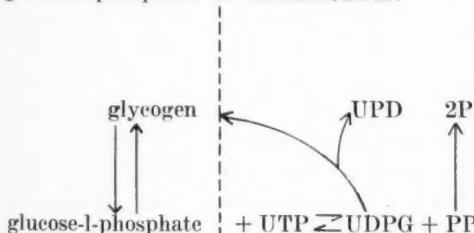
In the case of relaxing muscle, the equality would indicate either that the dissipation of work in relaxation is completely irreversible, or that in this equation the entropy term predominates, i.e., that the relaxing muscle is a fairly perfect rubber.

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Again, we have the fact that, when the muscle shortens, there is a liberation of an additional amount of heat, strictly proportional to the shortening  $\Delta L$ , which is called the shortening heat. Finally, there is the fact that any work that is being done is in addition to these two heat quantities: a muscle lifting a load will first develop the activation and shortening heat corresponding to the distance shortened, and the work  $F \times \Delta L$  is in excess of this, appearing as heat when, in relaxation, the muscle is stretched by the falling load. Thus, we can group the energies into two categories: the "overhead" needed for the activity of the mechanism, and the actual work added to this without further heat effects. These statements, which we owe primarily to the classical papers by Fenn<sup>1,2</sup> and Hill<sup>3,4</sup> can be summed up as:

$$\Sigma(\Delta H) = A + a \cdot \Delta L + W \quad (1)$$

In relaxation, apart from any external work being returned to the muscle, there is no measurable heat production or uptake; this lack is commonly regarded as evidence that relaxation is, energetically, passive and not coupled with chemical reactions. Such a view requires some elaboration because (apart from the fact that the thermoneutrality of relaxation may be due to a coincidental cancellation of the heat effects of several processes, which we cannot affirm or deny), if the activation of the working substance is exothermic, why is not the opposite process endothermic? A comparison may make this clearer. Let us consider the cyclical synthesis and breakdown of glycogen, pretending, with relatively small error, that the reversible transformation glycogen  $\rightleftharpoons$  glucose-1-phosphate is thermoneutral:



Here, the reactions on the left side are

reversible, although in practice, under actual circumstances in muscle, it would proceed in the direction of glucose-1-phosphate formation (which in our analogy will be compared with the contractile material at rest). To make glycogen (here representing active muscle), we have to couple the process with the exothermic break-down of UTP, in analogy with the activation of muscle by the breakdown of ATP (see below). Thus we can have a cycle in which glycogen synthesis ("activation of muscle") is exothermic, glycogen breakdown ("relaxation") about thermoneutral. The analogy breaks down, of course, among other things, because the active muscle can perform work and, when doing so, consumes more of the associated exothermic reaction. It suffices to illustrate the main point, however: activation is coupled with an exothermic biochemical process; relaxation is not, to our knowledge, coupled with anything. In more general terms, let us consider an energy-yielding reaction such as ATP breakdown, with its heat effect  $\Delta H$ , coupled with a transformation  $A \rightarrow B$  in activity, while the reversal  $B \rightarrow A$  takes place in relaxation without associated chemical reaction. Now, the laws of thermochemistry require that the total  $\Delta H$  of the energy-supplying reaction appear at the end (apart from unreversed external work), regardless of the specific nature of the  $A \rightarrow B \rightarrow A$  processes. But if  $A \rightarrow B$  and its reversal have themselves a finite heat effect, this would add itself to that of the coupled reaction in contraction, and appear with the reversed sign in relaxation. Hence, unless we are misled by a coincidence, the observations indicate that the transformations within the working substance, while coupled with an extraneous exothermic reaction, are approximately thermoneutral.

Since this conclusion will need considerable scrutiny, we shall not attempt to explore its full scope. It will merely be used to introduce the following problem: if the energy for each of the manifestations of muscular activity is derived from a coupled reaction, it would be important to establish a

complete biochemical energy balance for these events. Specifically: is it possible to assign definite amounts of chemical change with each of the three entities A,  $a \cdot \Delta L$ , and W, and are these entities associated with different reactions, or with separate quantities of the same reaction?

Before proceeding further, however, we must first decide what reactions are available. In previous periods, there has been much discussion about inogen, lactic acid, phosphorylcreatine and—more recently—K-Na exchange, and direct oxidative energization; but more and more compelling, if not entirely direct, evidence points toward the role of ATP as the primary energy donor. The foundation of the ATP theory is very strong, indeed (as I have expressed elsewhere<sup>5</sup>), it differs from all previous theories by the fact that it has a foundation. Yet, an assumption that is so basic requires direct experimental proof, and here we find ourselves in a peculiar state of uncertainty. Some years ago, Fleckenstein et al. and Davies,<sup>6,7</sup> and I<sup>8,9</sup> showed that after a rapidly interrupted contraction it was not possible to detect a diminution of ATP or of phosphorylcreatine (PC), which might have reversed a primary ATP breakdown. We should not regard this evidence as proof that the ATP theory is false. On the one hand, Carlson and Siger<sup>10</sup> have recently implied that our early experiments were simply in error; although I do believe that they were well performed, I also feel that a good deal more work is needed to elucidate the various aspects of the problem. On the other hand, there is a good possibility that, in such experiments, a primary breakdown of ATP may be concealed by a minor amount of another phosphoryl donor. We (as well as Davies et al.<sup>11</sup>) are now able to detect consistently a liberation of inorganic phosphate early in contraction of frog sartorius muscles. In our current experiments, this release of energy is correlated with an equivalent formation of creatine (in complete agreement with the ATP-PC theory); but in an earlier recent series the PC breakdown was not demonstrated, and

there was often a diminution of a highly labile phosphate compound, XP. Moreover, in our current series, XP is present but does not change; in the immediately preceding series XP was absent. It seems best to defer judgment until more experimental work has been done. Meanwhile, I should like to maintain, as did Hill<sup>12</sup> in 1950, that a concept of such paramount significance cannot be accepted as final until it is based upon direct proof.

It is clear, on the other hand, that during more prolonged activity, e.g., in a series of twitches or of brief tetani, a breakdown of PC does occur. This breakdown of PC presumably reflects a primary breakdown of ATP, although, strictly speaking, this relationship has not yet been shown to apply to living muscle, since the ATP does not decrease until after a sizeable decrease in PC. Without belittling the primary role of ATP, we shall present our work in terms of PC breakdown, because that is what we measure and because it is this breakdown that contributes to PC reaction heat. It is best demonstrated with iodoacetate-poisoned muscles studied anaerobically, because in such muscles there is no possibility of a resynthesis of PC. The following reactions may then occur:



Dependent upon the circumstances, reaction (2) or (3) may predominate. Work at low temperature suppresses (3) and this is advantageous because, once phosphorylation of glycogen occurs with the P formed in reaction (2), there may also be further phosphorylation of fructose-6-phosphate at the expense of more PC; the latter reaction is not connected with mechanochemical activity and therefore contributes an error to the measurement of the PC that is broken down and mechanically utilized. Reaction (3) is not always fully suppressed at 0 C.: in an extensive series in which it was not suppressed we could establish that its occurrence is not correlated with shortening and work. In other experiments that showed only re-

action (3), shortening and work went on just as well. This leads to the first conclusion: variations in energy produced in the three categories A,  $a \cdot \Delta L$ , and W, are not derived from different reactions, but all result from the breakdown of PC according to reaction (2). The question is then: can we detect various parcels of PC breakdown that correspond quantitatively with A,  $a \cdot \Delta L$ , and W?

We have devoted a great deal of time to the following experiments: of each pair of muscles, one served as the resting control, the other performed, e.g., 12 tetanic contractions against a certain load; the shortening  $\Delta L$  was measured, and the PC breakdown determined from the difference in composition. A number of such results were then plotted as chemical change per gram per contraction against the shortening per length. If the results would conform to equation (1), a plot as in figure 1, curve A would result. The actual findings displayed a dismaying amount of scatter, and their graphic representation supplied a perfect illustration of Fisher's<sup>13</sup> dictum: "Diagrams prove nothing, but bring outstanding features readily to the eye; they are therefore no substitute for such critical tests as may be applied to the data, but are valuable in suggesting such tests, and in explaining the conclusions founded upon them." Such critically designed experiments are now in progress, but at this early stage we shall have to see what we can do with the older data.

One thing is quite clear at the present stage: muscles contracting against a moderate load, and so doing about optimal isotonic work, decompose more PC than do muscles that are not performing work. Hence, the biochemical counterpart of the "Fenn effect," the factor W in equation (1), seems to have been demonstrated, although I would postpone a quantitative discussion until the termination of properly designed and evaluated paired experiments. Also, we find that at zero shortening, the activation metabolism A is of the order of 0.3 micromoles per gram per contraction, in good agreement with our

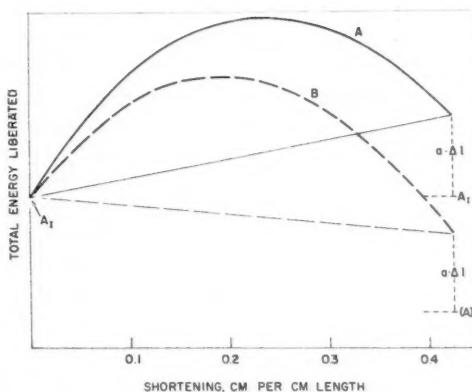


Figure 1

Diagram of the possible dependence of the total energy liberation upon the shortening of stimulated muscle, as determined by the isotonic load. In curve A, the energy at any given degree of shortening consists, as in formula (1), of the activation heat  $A_1$ , the shortening heat  $a \cdot \Delta L$ , and the work W. In curve B,  $a \cdot \Delta L$  and W appear as before, but the activation heat  $(A)_T$  is variable and diminishes with the resulting length of the muscle.

original estimates<sup>14</sup> and with other recent experiments.<sup>10</sup> But as to the factor  $a \cdot \Delta L$ , the situation is less satisfactory. Not only was the variation of the points too great to estimate the actual course of the curve, but it appeared that, in the region of maximal shortening, the scatter exceeded the limits that could reasonably be expected from the variability between two muscles of a pair. Indeed, direct comparisons between maximal and small shortening (with roughly the same amount of work) even showed that the shortening muscles in certain series decomposed less creatine than the nonshortening ones, contrary to expectation and contrary to the results of larger experimental series.\*

While further direct experimentation along these lines will establish the direct biochemical evidence, some insight already exists into the reason for the variable results. First,

\*This unexpected result, on the other hand, seems to have been the rule in the parallel work by F. D. Carlson of Johns Hopkins University. Neither his nor our work has been published, but both have mentioned these matters at several scientific meetings.

in equation (1), we assumed that  $A$  is a constant. However, we know from Aubert's work<sup>15</sup> that this is not the case: in addition to being determined by a time factor that we have assumed to be constant,  $A$  depends on the length, or primarily upon the tension of the muscle;\* and while over certain ranges (such as those preferably employed in Hill's 1938 study<sup>3</sup>) the variation of  $A$  is small,  $A$  is, in general, much less in a muscle that is shortened to well below the natural length. Therefore, we must write:

$$\Sigma(\Delta H) = (A)_T + a \cdot \Delta L + W. \quad (4)$$

Curve A in figure 1 can then only occur if, in a special situation,  $A$  is constant. If, on the other hand, the change in  $A$  upon shortening is considerable (or if  $a$  is less than usual), a maximally shortening muscle may well produce less heat, and engage in less metabolism, than an isometrically contracting one:

$$A_I > (A)_T + a \cdot \Delta L \quad (5)$$

In such case we would obtain curve B of figure 1. The literature is not explicit in this respect. According to Hill,<sup>3</sup>  $A$  does not seem to vary greatly in the experiments on which the concepts were derived (but see Hill<sup>3</sup> pp. 169-170), but recent calorimetric results by Tsigi,<sup>16</sup> and a sleight-of-hand extrapolation one can make of some of Fenn's<sup>1</sup> curves, as well as the data of Nachmansohn (Tables X and XI<sup>17</sup>), are in accordance with equation (5). Our own exploratory

\*In making this distinction between length or tension as the determinant variable, I am not alluding to the inquiry whether muscle is primarily a tension- or a shortening-generator, since I do not know if this is a valid question. The distinction appears when we try to estimate the maintenance heat of a muscle during shortening or stretch. Should one study the change in length from point to point and take the corresponding value of  $A$  from an empirically determined plot of  $A$  against  $L$ ? Or, should one take the  $A$  associated with the prevailing tension (which in turn depends on the velocity  $dL$ )? In Aubert's experiments,  $A$  depends linearly on the tension; this relationship is one of the reasons why we tend to favor the latter decision. Whatever the final decision, it is clear that, with respect to the maintenance of heat, the distinction assures a real operational meaning.

results show that the quantitative relationships may differ from muscle to muscle, although more instances, so far, have been in the direction of curve A than of curve B. Clearly, there can be any number of intermediate cases between the extremes of equations (1) and (5). Therefore, it is likely that our erratic results, and those with the opposite tendency of Carlson, are in full agreement with equation (4), meaning that each of the terms: activation, shortening, and work energy, are associated with a definite quantity of the same overall reaction:  $PC \rightarrow P + C$ . But the accurate establishment of these relationships (including the parallel, but not necessarily symmetrical, case of negative work) will still require a great deal of painstaking work.

Believing that there should be a balance between experimental and theoretical work, I am not tempted at this moment to speculate on the mechanisms by which the muscle determines how much biochemistry to call upon during a given act of activity. There are many facets to this. On the one hand: what causes metabolism to be so greatly intensified? It is not entirely a matter of its being irrevocably coupled with the mechanical change that is elicited by stimulation, because it is known that metabolism can be greatly accelerated, without a corresponding increase in mechanical activity, by raising the external  $K$  concentration (an effect that requires the presence of  $Ca$ , just as excitation-contraction coupling does<sup>18</sup>). On the other hand, what determines the quantity of energy that is mobilized? That the activation energy depends on length and that a shortening energy does occur are not beyond imagination, since changes in length do involve a change in some configuration. Although it is harder to visualize the mobilization of extra energy for work, even the mystery surrounding this problem might disappear if it were formulated in different terms, e.g., relating the rate of energy production to the velocity and extent of shortening.

Finally, it may be stated that, while the present uncertainties, not only of the experi-

mental results but even of the theoretical expectations, may be disturbing, it has its tranquilizing aspects as well. A good deal of thought has probably been given lately to the problem of harmonizing certain properties of the myocardium (e.g., the lower "efficiency" when working against high pressure, i.e., at lesser shortening, than when the same work is done by ejecting a large volume of blood against a low resistance) with the "Hill laws" formulated in equation (1); these efforts have largely remained unpublished, probably because they were unsuccessful. The independent variability of the parameters of equation (4) enable us to treat such different cases by the same approach; this independence also suggests that the separate study of A as a function of the conditions, and of a and W, in conjunction with other dynamic quantities and with their metabolic correlates, will be a rich field for future investigation.

So viewed the present status of the field is not really disappointing; it is merely that we have looked upon the matter from too simple a viewpoint. The inability to obtain prompt answers has, at least, caused the problems to be formulated more realistically.

#### Acknowledgment

The biochemical investigations alluded to in this presentation have been carried out during the last four years in cooperation with K. Seraydarian and A. Wallner. M. O. Schilling has, meanwhile, developed our instrumentation, along the lines of A. V. Hill's methodology, for the measurement of heat production in muscle. During the few months since, our explorations in myothermic work have been carried out by Dr. B. C. Abbott (Department of Zoology, The University of California, Los Angeles) and Professor S. L. Dart (Department of Physics, Claremont College for Men, Claremont, California, on leave in our laboratory).

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## High Energy Phosphates and the Force of Contraction of Cardiac Muscle

By ROBERT F. FURCHGOTT, PH.D., AND KWANG SOO LEE, M.D., PH.D.

This paper reviews studies performed by the authors and by others on the levels of high energy phosphate compounds—adenosine triphosphate (ATP), adenosine diphosphate (ADP), and creatine phosphate (CP)—of cardiac muscle under various conditions influencing contractile force. Interference with energy metabolism decreases both contractile force and high energy phosphates, especially CP. Certain types of experimental "failure" are associated with decreases in high energy phosphates; however, other types of "failure" occur without significant decreases in these phosphates. In addition, marked decreases or increases in contractile force, independent of significant changes in high energy phosphates, can be produced by drugs, by changes in heart rate, or by alterations in extracellular concentrations of cations. A decrease in force when levels of high energy phosphate are normal may be attributed to a deficiency in the utilization of these energy stores for mechanical work. The nature of this deficiency has been analyzed in isolated cat papillary muscles by simultaneously determining the activity oxygen consumption and contractile force per beat. In the case of decreases in contractile force due either to reduced heart rate or to spontaneous heart failure, the deficiency may be attributed almost completely to a loss in efficiency in the conversion of chemical energy in the high energy phosphates to mechanical energy (work). Cardiac glycosides, in restoring contractile force, do so by restoring the efficiency of this conversion. In the case of decreases in contractile force resulting from lowered extracellular  $\text{Ca}^{++}$ , the deficiency may be attributed partly to a loss of efficiency in this conversion and partly to a reduction in amount of high energy phosphate utilized per beat.

IT IS NOW generally agreed that the energy for muscle contraction comes either directly or indirectly from the splitting of high energy phosphate bonds. Although there may be other compounds that account for a small percentage of the total high energy phosphate bonds of cardiac muscle, it is reasonably certain that 90 per cent or more of such bonds occur in adenosine triphosphate (ATP) and creatine phosphate (CP).<sup>1, 2</sup> In cardiac muscle under steady-state conditions, it may be assumed that the rate of utilization of high energy phosphate bonds for mechanical work and for other processes in which free energy is required is balanced by the rate of resynthesis of high energy phosphate

bonds as a result of the coupling of phosphorylation with metabolism of food stuffs, primarily oxidative metabolism. If the energy for contraction is derived from high energy phosphate bonds, then it might be expected that some correlation could be found between the level of the principal high energy phosphate compounds in heart muscle, namely ATP and CP, and the strength of contraction. The first part of this paper will deal with the results of experiments carried out in the laboratories of the authors, as well as in other laboratories, to investigate the relationship between high energy phosphate content and the contractile strength of cardiac muscle. The inescapable conclusion to be drawn from these results is that under many conditions which markedly alter the contractile strength of heart muscle there is no corresponding alteration in the levels of high energy phosphate compounds. These results have led us<sup>3, 4</sup> to postulate, as Wollenberger<sup>5</sup> did previously, that decreases in strength of cardiac contraction under many experimental conditions are not due

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Table 1

*Concentrations of Inorganic Phosphate, Creatine Phosphate, and Adenine Nucleotides in Mammalian Cardiac Muscles*

Cardiac tissue analyzed	Concentration in $\mu\text{M}/\text{Gm. tissue}$					Reference
	IP	CP	ATP	ADP	AMP	
Dog left ventricle (apex) in situ	1.9	12.8	5.7	—	—	Wollenberger et al., 1960 <sup>2</sup>
Dog left ventricle of HLP	5.26 <sup>†</sup>	7.62 <sup>†</sup>	4.3 <sup>*</sup>	—	—	Fawaz and Tutunji, 1957 <sup>13</sup>
Cat ventricle (apex) in situ	5.7 <sup>†</sup>	5.2 <sup>†</sup>	3.39	0.69	0.19	Fleckenstein et al., 1959 <sup>8</sup>
Cat papillary muscle in vitro	3.2	7.04	3.81	0.22	0.17	Lee et al., 1961 <sup>10</sup>
Rat ventricle in situ	3.8	6.8	4.12	0.71	0.53	Fleckenstein et al., 1958 <sup>8</sup>
Rabbit left ventricle in situ	1.9	8.2	—	—	—	Wollenberger et al., 1960 <sup>2</sup>
Guinea-pig left ventricle in situ	2.0	10.9	—	—	—	Wollenberger et al., 1960 <sup>2</sup>
Guinea-pig left ventricle in situ	3.71	8.38	5.59	0.64	0.34	Feinstein, 1960 <sup>7</sup>
Guinea-pig left ventricle in situ	1.66	7.20	3.48	1.02	—	Hochrein and Döring, 1958 <sup>9</sup>
Guinea-pig left ventricle, HLP	2.78	7.01	3.15	0.88	—	Hochrein and Döring, 1958 <sup>9</sup>
Guinea-pig left atrium in vitro, 37°	6.24	4.19	2.41	0.43	0.23	Furchtgott and de Gubareff, 1958 <sup>8</sup>
Rabbit atria in vitro, 30°	4.3	3.9	2.21	0.28	0.31	Fleckenstein et al. 1959 <sup>8</sup>

\*Estimated from acid-labile phosphate, assuming 90% arises from terminal phosphates of ATP.

†Figures probably reflect some conversion of CP to IP during freezing procedure.

to a deficiency of high energy phosphate stores but to an impairment or deficiency in the utilization of such stores for contraction.

Deficiency in utilization of high energy phosphates for contraction may result from a lower rate of utilization per contraction at essentially normal efficiency for conversion of phosphate bond energy into mechanical energy; an essentially normal rate of utilization per contraction at decreased efficiency; or a combination of these 2 conditions. The last section of this paper will be directed at the problem of the nature of the deficiency in the utilization of chemical energy for contraction under certain experimental conditions that lead to marked alterations in strength of contraction with small or insignificant changes in high energy phosphate content.

#### Levels of High Energy Phosphate Compounds Under Control Conditions

Over the past 10 years the reported levels for high energy phosphate compounds in cardiac muscle, especially that of CP, have risen considerably. This is in large part due to the development of more refined procedures for the determination of these compounds—with better methods being applied not only in the analyses of extracts of cardiac muscle but also in the quick-freezing of the

tissues and in the extraction of powders made from the frozen tissues.<sup>1-3, 6-9</sup> Table 1 presents data obtained by ourselves and a number of other investigators with procedures that we feel give reasonably accurate values for the steady-state levels of CP and ATP, as well as of inorganic phosphate (IP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Some of these values apply to hearts in situ, some to heart-lung preparations (HLP), and some to isolated, beating cardiac preparations in vitro. All of the preparations were under control conditions in the sense that contractile strength was at a steady level and there was no indication of "failure."

Table 1 shows that ventricular muscle CP ranges from about 7 to 12 micromoles/gram and ATP from about 3.5 to 5.5 micromoles/gram. The levels of ADP are much lower than those of ATP, ranging from about one-fourth to one-twentieth of the latter in the different preparations. The differences in the levels of both ATP and CP reported by different authors probably are due in part to the use of somewhat different procedures and in part to actual differences in levels among species. It would appear that in heart-lung preparations and in isolated papillary muscle in good physiologic condition, the

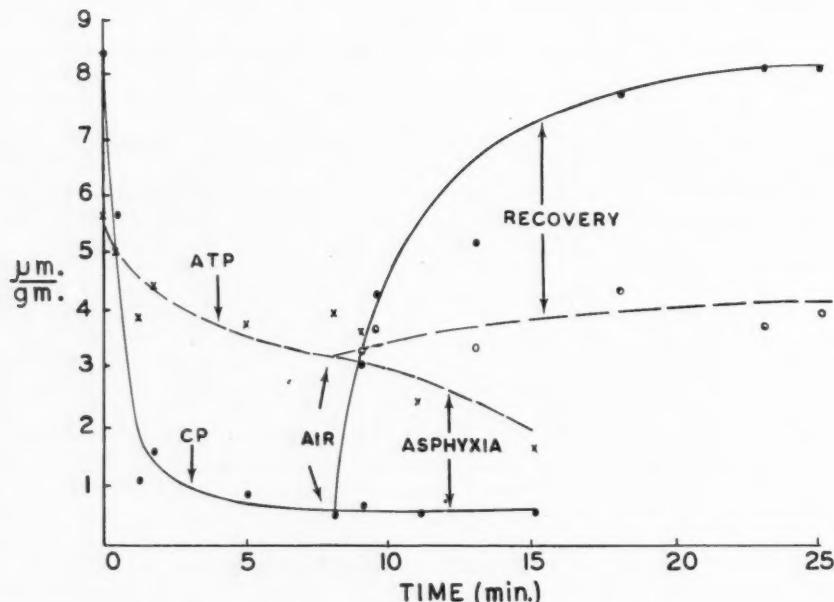


Figure 1

The effect of acute asphyxia and subsequent recovery from asphyxia on creatine phosphates and ATP in guinea-pig heart *in situ*. Each pair of points at any given time represents levels in a single animal, except for pair of points at zero time, which represents mean levels in control animals. Asphyxia was started at zero time. Continuous falling curves are fitted to points obtained with 8 animals subjected to varying periods of asphyxia. Rising curves are fitted to points obtained with 6 animals after varying periods of recovery after resumption of artificial respiration ("air") after 8 minutes of asphyxia. (Data of Feinstein<sup>7</sup> used with his permission.)

levels of CP and ATP are essentially the same as those found in the heart *in situ*<sup>9-11</sup> in any given species.

The ATP and CP levels in atria (table 1) are considerably lower than those in ventricles in the same species. For example, in our laboratory, we have found that isolated atria of guinea pigs contain only about half as much CP and ATP as do ventricles *in situ*.<sup>3, 7</sup> This smaller content of high energy phosphate compounds in atrial muscle is not too surprising in view of the findings of earlier workers that the acid-labile phosphate (mainly from ATP) of atrial muscle is only about one-half of that of ventricular muscle in the rabbit and dog.<sup>12-14</sup>

#### Influence of Inhibition of Oxidative Metabolism

Conditions or agents that interfere with oxidative metabolism would be expected to

lower the level of high energy phosphate in cardiac muscle if the rate of utilization of these compounds for contraction and other processes requiring free energy exceeded the rate of their resynthesis by the inhibited metabolism. It is, therefore, not surprising that the high energy phosphate levels fall markedly below control levels when cardiac muscle is subjected to a lack of oxygen or to agents that inhibit oxidative metabolism, as shown in table 2. In all cases in which metabolism is impaired, there is also a very marked reduction in contractile strength.

Note that in every case the fall of CP is greater than that of ATP, and that in some cases a marked fall in CP is accompanied by only a small or insignificant fall in ATP. The fact that CP is much more sensitive to impairment of metabolism than is ATP was

Table 2

*Changes in High Energy Phosphates Accompanying Decreases in Contractile Strength Produced by Impairment of Energy Metabolism*

Condition or agent impairing energy metabolism	Type of cardiac preparation	% Change from control level			Reference
		Contractile strength*	CP	ATP	
Anoxia for 18 min.	Guinea-pig atrium in vitro	-93	-69	-7	Furchtgott and de Gubareff, 1958 <sup>a</sup>
Anoxia for 30 min.	Cat papillary muscle in vitro	-78	-77	-51	Lee et al., 1961 <sup>10</sup>
Asphyxia for 8 min.	Guinea-pig ventricle in situ	>-70	-94	-32	Feinstein, 1960 <sup>7</sup>
Hypoxia (4% O <sub>2</sub> ) for 30 min.	Rat ventricle in situ	Marked fall	-70	-21	Fleckenstein et al., 1959 <sup>8</sup>
Dinitrophenol	Dog ventricle, HLP	-62	-63	0†	Fawaz and Tutunji, 1957 <sup>11</sup>
Fluoroacetate, 15 mg.	Dog ventricle, HLP	-78	-59	-12†	Fawaz, 1956 <sup>11</sup>
Phenylbutazone	Guinea-pig ventricle, HLP	Marked fall	-50	-35	Hochrein and Döring, 1958 <sup>9</sup>

\*Indices of contractile strength: contractile amplitude for guinea-pig atrium; contractile force for papillary muscle; "maximal isometric pressure" for guinea-pig ventricle in situ; cardiac output for heart-lung preparations.

†Estimated from acid-labile phosphate, assuming 90% arises from terminal phosphates of ATP.

also indicated in earlier work on the effect of anoxia and metabolic inhibitors on the labile phosphates of cardiac muscle.<sup>12, 15-17</sup>

From the results in table 2 it would appear that, under adverse metabolic conditions, decreases in the strength of contraction are more closely related to decreases in CP than to decreases in ATP. In this connection the results of some experiments by Maurice Feinstein,<sup>7</sup> obtained while he was a graduate student in our department, are worth citing. In these experiments he produced asphyxia in open-chest guinea pigs by tracheal occlusion for varying periods. In some of the animals he reinstated artificial respiration after 8 minutes of asphyxia in order to follow the process of recovery. Figure 1 shows his findings on the levels of ATP and CP at various times after the onset of asphyxia and also at various times during the recovery from asphyxia. The fall in CP was much more rapid and extreme than the fall in ATP. Moreover, after reinstatement of artificial respiration, CP returned to essentially the control level within about 10 minutes, whereas ATP showed only a slight recovery over a period of almost 20 minutes. In most of the animals used, the left intraventricular pulse

pressure was continuously followed over the course of the experiment, and in some animals the "maximal isometric pressure" that the left ventricle could produce was determined at intervals by temporary complete constriction of the ascending aorta. Using "maximal isometric pressure" as an index of contractile strength, it appeared that contractile strength was close to normal as long as the level of CP was at least 20 to 30 per cent of the control level, but that decreases of CP to still lower levels were associated with marked decreases in contractile strength. In the recovery period, after reinstatement of artificial respiration, contractile strength was essentially back to normal by the time (less than 1 minute) CP had been restored to about 30 per cent of the control level, even though there was no increase in ATP above its depressed level within the same period.

From his experiments on cardiac asphyxia *in situ* Feinstein concluded that there was no correlation between decreases in contractile strength and decreases in ATP content, but a fairly good correlation between decrease in strength and extreme decrease in CP content. Hochrein and Döring,<sup>9</sup> on the basis of their results with guinea-pig heart-

Table 3

*Changes in High Energy Phosphates Accompanying Changes in Contractile Strength Produced by Drugs or Alterations of Experimental Conditions*

Drug or experimental condition	Type of preparation	% Change from control level*		
		Contractile strength	CP	ATP
High $\text{Ca}^{++}$ in medium	G.P. atrium	+310	0	0
	Cat pap. m.	+160	0	-25‡
Low $\text{K}^{+}$ in medium	G.P. atrium	+ 70	-20	-13‡
	Cat pap. m.	+ 89	-39	-37
Epinephrine (low)	G.P. atrium	+210	0	0
	G.P. atrium	+335	-19	0
Cardiac glycoside (max. inotropic level)	G.P. atrium†	+605	0	0
	Cat pap. m.†	+500	0	0
Decreased frequency (from 60 to 6 per min.)	G.P. atrium	- 60	0	0
Low $\text{Ca}^{++}$ in medium	Cat pap. m.	- 86	0	0
Acetylcholine	G.P. atrium	- 94	0	0
Ryanodine	G.P. atrium	- 90	0	0
Cardiac glycoside (toxic level)	G.P. atrium	- 57	-64	-23
	Cat pap. m.	- 84	-66	-42

\*Zero denotes no statistically significant change.

†After spontaneous failure. In these experiments levels in failure were used as control levels.

‡Borderline statistical significance.

lung preparations (especially those poisoned with phenylbutazone and fluoroacetate), also came to the conclusion that there was a good correlation between decrease in the work capacity of the heart (as determined by the "competence index") and the decrease in content of CP, rather than that of ATP. The results of these investigators, as well as those of others shown in table 2, suggest that CP may be more directly involved than ATP in supplying energy for contraction. However, it should be emphasized that all of these results were obtained on cardiac preparations in which oxidative metabolism was impaired experimentally, and that other changes produced by impairment of metabolism—such as decreases in intracellular pH, alteration in intracellular content of various ions, and increases in intermediary metabolites—may have been more responsible for the decreases in contractile strength than the changes in CP. (See also comments by Fawaz and Tutungi<sup>18</sup> on the lack of correspondence between cardiac output and CP levels in well-oxygenated heart-lung preparations poisoned with dinitrophenol.)

#### Influence of Alterations in Experimental Conditions Which Do Not Inhibit Oxidative Metabolism

A large number of alterations in experimental conditions, none of which is thought to act primarily by interfering with oxidative metabolism, can produce marked increases or decreases in the contractile strength of cardiac muscle. Among these are alterations brought about by additions of certain drugs, by changes in the ionic content of the extracellular fluid, and by changes in frequency of contraction. In our laboratories we have investigated the levels of high energy phosphates of 2 isolated, electrically driven cardiac preparations—namely, the guinea-pig left atrium and the cat papillary muscle—subjected to some of the alterations that influence contractile strength.<sup>3, 4, 10</sup> Our findings are shown in table 3. In all cases in which similar alterations in experimental conditions were used on both preparations, the results obtained were essentially the same.

The upper part of the table shows the results obtained under experimental conditions that produced increases in contractile strength. The only condition of this type

Table 4

Changes in High Energy Phosphates Associated with Experimental Failure

Type of preparation	Procedure for producing failure	% Change from control level*			Reference	
		CP	ATP	ADP		
Guinea-pig left atrium <i>in vitro</i>	"Spontaneous"	0	0	0	0	Furchtgott and de Gubareff, 1958 <sup>3</sup>
Cat papillary muscle <i>in vitro</i>	"Spontaneous"	0	0	—	—	Lee et al., 1960 <sup>4</sup>
Dog HLP	"Spontaneous"	+36	0	—	—	Wollenberger, 1947 <sup>20</sup>
Guinea-pig HLP	Extreme volume-loading	-32	-11†	+16†	—	Hochrein and Döring, 1958 <sup>9</sup>
Rat heart <i>in situ</i>	Aortic constriction (acute failure 30 min.)	-57	-16	—	—	Szekeres and Schein, 1959 <sup>22</sup>
Guinea-pig heart <i>in situ</i>	Aortic constriction (chronic failure)	-54	-24	-40	0	Feinstein, 1960 <sup>7</sup>
Dog heart <i>in situ</i>	Tricuspid valve avulsion plus pulmonary artery stenosis (chronic failure)	0	0	—	—	Olson and Piatneck, 1959 <sup>23</sup>

\*Zero denotes no statistically significant change.

†Average of only 2 experiments. Significance doubtful.

under which there was an appreciable change in the level of high energy phosphates was that in which the  $K^+$  concentration of the bathing medium was drastically reduced, and then the change was a decrease rather than an increase. All of the other experimental conditions (elevation of  $Ca^{++}$  concentration of the medium and addition of catecholamines or cardiac glycosides) led to marked increases in strength with small or insignificant changes in the levels of CP and ATP. Our results with cardiac glycosides are in confirmation of Wollenberger's earlier results on the dog heart-lung preparation.<sup>19</sup>

The lower part of table 3 shows the results obtained under experimental conditions that produced decreases in contractile strength. It is noteworthy that the reductions in strength brought about by varied procedures, such as decrease of frequency of stimulation, decrease of extracellular  $Ca^{++}$ , addition of acetylcholine, and addition of ryanodine, were accompanied by no significant change of CP and ATP from control levels. Previous to our work Wollenberger<sup>20</sup> and Fawaz and Hawa<sup>11</sup> had already found that local anesthetics and pentobarbital were

able to impair the contractile strength of the dog heart-lung preparation markedly without producing a significant fall in the high energy phosphate content of the ventricular muscle.

The fall in contractile strength produced by cardiac glycosides at the toxic dose level was accompanied by significant decreases in both CP and ATP. However, this is not too surprising in view of the recent findings of Lee et al.<sup>21</sup> that mitochondria obtained from hearts poisoned with a cardiac glycoside appear to have some degree of uncoupling of oxidative phosphorylation. Thus, the condition produced with toxic levels of cardiac glycosides may be an interference with oxidative metabolism, which would be expected to lower the concentrations of high energy phosphates.

The results shown in table 3 strikingly demonstrate that the strength of contraction of cardiac muscle may vary widely despite essentially constant levels of high energy phosphates. The decreases in strength as one goes from those experimental conditions that produce marked positive inotropic effects to control conditions, and from control conditions to those that produce marked negative

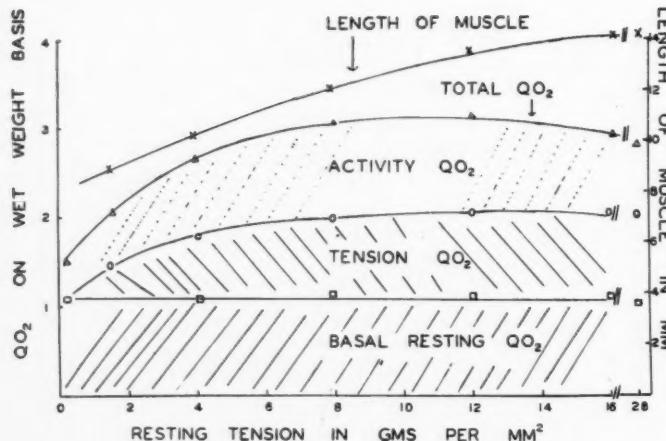


Figure 2

The effect of resting tension on the oxygen consumption of a papillary muscle stimulated at a frequency of 60 per minute. Basal resting  $Q_O_2$  represents  $O_2$  consumption of the unstimulated muscle at zero tension. Tension  $Q_O_2$  represents the increment in consumption resulting from application of tension on unstimulated muscle. Activity  $Q_O_2$  represents the increment in consumption owing to the contractile activity after stimulation at a given resting tension. (From Lee.<sup>25</sup>)

inotropic effects, must therefore be attributed to deficiencies or impairments in the utilization of the available high energy phosphate stores for contraction.

#### Influence of Experimental Heart Failure

Three types of preparations have been used for concomitant studies of mechanical failure and high energy phosphate compounds in heart muscle. One type is the isolated cardiac preparation, such as the papillary muscle of the cat or the left atrium of the guinea pig, suspended in a physiologic medium and driven at a constant rate by electric stimulation. Such preparations will undergo spontaneous failure, as indicated by a loss of contractile force, in the course of several hours. A second type is the heart-lung preparation, which also will undergo failure spontaneously over a period of several hours, even when there is no change in aortic resistance or venous reservoir pressure during the course of an experiment. This second type of preparation can also be made to go into failure at a faster rate by increasing the venous return (extreme volume-loading) or at still a faster rate by increasing the aortic resistance. A third type of preparation is that in which failure is brought about *in situ*, either acutely or chronically, either by constricting the aorta or by constricting the pulmonary artery along with avulsion of the tricuspid valves. Chronic failure brought about by these

procedures produces changes in the whole animal very similar to those seen in patients with congestive heart failure.

Table 4 gives the results of several investigations carried out with these different types of preparations. Acute spontaneous failure occurred in the isolated cardiac preparations<sup>3, 4</sup> and in the dog heart-lung preparation<sup>18</sup> with no decrease in high energy phosphate levels. On the other hand, acute failure in the guinea-pig heart-lung preparation brought about by extreme volume-loading,<sup>9</sup> and acute failure of the rat heart *in situ* produced by marked constriction of the aorta<sup>22</sup> were both associated with significant falls in high energy phosphates. In chronic heart failure initiated by stress on the right side of the heart, Olson and Piatneek<sup>23</sup> found no significant change in levels of high energy phosphates. However, in chronic heart failure initiated by stress on the left side of the heart, Feinstein did record significant decreases.<sup>7</sup>

In those cases of failure in which there was no significant change in high energy phosphate levels, there is again a clear dissociation of contractile strength and useful energy stores. Such cases of failure have therefore been attributed to a deficiency or impairment in the utilization of high energy phosphate stores for mechanical work.<sup>3-5, 23, 24</sup> In the case of acute failure in the guinea-pig

heart-lung preparation with extreme volume-loading, and in the rat heart *in situ* with aortic constriction, we feel that the fall in high energy phosphates may be largely due to the demand for oxygen to support an increased work load, that exceeds the supply of oxygen delivered through the coronary circulation—thus, leading to an adverse metabolic condition in the heart muscle.

In chronic failure in guinea pigs, studied by Feinstein in our laboratories, a number of typical signs of marked congestive failure were apparent, including cardiac hypertrophy, pulmonary edema, elevated venous and right ventricular pressures, and elevated left diastolic pressures. Thus, it would appear that in chronic congestive failure associated with aortic stenosis, there may be a considerable fall in high energy phosphate level. Again, the average fall of CP was much greater than that of ATP in these animals, and there was a fair correlation between the severity of failure (estimated on the basis of physiologic and pathologic changes) and the extent of reduction of CP. A second finding which should be mentioned and which was made by another graduate student in our department, Arnold Schwartz, was that the efficiency of oxidative phosphorylation (determined by P:O ratios) of mitochondria from guinea-pig hearts in congestive failure was depressed about 30 to 40 per cent below that of mitochondria from normal guinea-pig hearts. Thus, there is a possibility that the low levels of high energy phosphates in guinea-pig hearts in congestive failure may be due in part to a loss in efficiency of mitochondrial oxidative phosphorylation.

The findings of Feinstein and Schwartz do indicate that an impairment of synthesis and a decrease in the steady-state levels of high energy phosphate bonds may contribute to the severity of chronic congestive failure of guinea pigs with aortic constriction. However, it is impossible at present to state whether these changes played a primary role in producing the state of circulatory failure or whether they themselves developed only after the onset of physiologic and biochemical

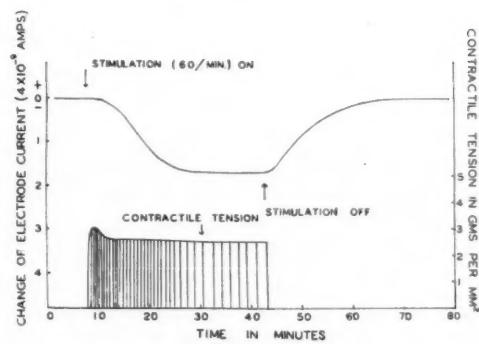


Figure 3

*Changes in electrode current and contractile tension before, during, and after electrical stimulation of a papillary muscle. Muscle diameter, 0.32 mm.; wet weight, 3.8 mg.; tension, 2 Gm./mm.<sup>2</sup>. Electrode current measures  $O_2$  tension in effluent from muscle. The change in the current produced by stimulation can be used directly for calculating the rate of activity oxygen consumption. (From Lee.<sup>25</sup>)*

changes resulting from failure of the overloaded heart to maintain an adequate output. Feinstein, himself, was able to show that the impaired function of the failing heart was not strictly dependent on the decreased CP content. By injecting ouabain into guinea pigs in congestive failure he was able to improve cardiac function markedly and acutely (as judged from changes in intraventricular pulse pressures) even though the cardiac glycoside caused no increase in either CP or ATP above the low levels found in untreated animals.

#### Changes in Efficiency in the Utilization of High Energy Phosphates for Contraction

In the 2 previous sections many examples of alterations in the contractile strength of cardiac muscle without significant alterations in high energy phosphate stores have been cited. This type of finding has led to the conclusion that a decrease in contractile strength in one experimental condition as compared with another often results from a relative deficiency or impairment of the utilization of available high energy phosphates. However, the question is still left open of whether such a deficiency represents (1) a

decrease in the utilization of high energy phosphate bonds per contraction, with little change in the efficiency of the conversion of the chemical energy in these bonds to mechanical energy; (2) a decrease in the efficiency of the conversion of chemical to mechanical energy with little change in the utilization of high energy phosphate bonds per contraction; or (3) a combination of both situations. To obtain a complete answer to this question one would have to determine simultaneously the strength of contraction and the amount of high energy phosphate bonds used in each contraction of the cardiac muscle under different experimental conditions. At present, however, there is no available method for determining directly the amount of high energy phosphate bonds used in each contraction, and, therefore, an indirect approach must be employed.

The indirect approach developed by one of us (K.S.L.) involves the use of the isolated, electrically driven cat papillary muscle in an experimental set-up that permits the simultaneous measurement of contractile strength (usually isometric contractile tension) and rate of oxygen consumption (calculated from the fall in oxygen tension registered with a platinum "oxygen electrode" in a continuous-flow system).<sup>25</sup> With this set-up one can first obtain the "resting" oxygen consumption of the unstimulated, quiescent muscle at a fixed resting tension and then the "total" oxygen consumption when the muscle is stimulated at the desired frequency at the same resting tension. The difference between the rates for total and resting oxygen consumption gives the rate of extra oxygen consumption required for contractions under the given experimental condition. From this rate and the frequency of contraction one can readily calculate the extra oxygen consumed per beat.

Bing<sup>26</sup> has stressed the need for using the activity oxygen consumption of cardiac muscle rather than the total oxygen consumption, if one wishes to calculate the chemical energy used for the contraction process only. However, to obtain the activity oxygen consump-

tion of the intact heart (or of the left ventricle) one must be able to determine its oxygen consumption in the arrested state, with good coronary circulation and with intraventricular volume maintained at the mean diastolic volume of the active heart. These requirements are extremely difficult to meet; and the best procedure so far used for arresting the intact heart—namely, that of markedly increasing the  $K^+$  concentration of the blood<sup>27</sup>—is open to some criticism.

In contrast to the experimental difficulties hindering the determination of activity oxygen consumption in the intact heart is the ease with which it can be determined in the isolated cat papillary muscle. Figure 2 shows both the resting and the activity oxygen consumption of cat papillary muscle, driven at a frequency of 60 per minute, as a function of resting tension.\* Since the resting oxygen consumption, which is the sum of the consumption at zero tension ("basal resting" oxygen consumption) and the extra consumption resulting from the application of tension ("tension" oxygen consumption), considerably exceeds the activity oxygen consumption at all tensions, it is apparent that the use of total oxygen consumption for the estimation of chemical energy required for mechanical work will give values that are much too high.

Figure 3 is a plot of data from a typical experiment in which the activity oxygen consumption and the contractile tension of a papillary muscle were determined simultaneously. The difference between the steady-state level of the oxygen-tension curve during rest and during contraction at a fixed frequency can be used directly for calculations of the rate of activity oxygen consumption. By dividing the activity oxygen consumption per beat into the contractile tension at the steady-state level, one can obtain an index of the mechanical efficiency of the cardiac muscle under the experimental condition used.<sup>25</sup>

\*Figures 2 and 3 reproduced from Lee: *J. Physiol.* 151: 186, 1960.<sup>25</sup> By permission of the Journal of Physiology.

Table 5

*The Effect of Resting Tension on the Mechanical Efficiency of a Cat Papillary Muscle at Constant Frequency (60 per Minute)*

Resting tension (Gm./mm. <sup>2</sup> )	Activity $O_2$ uptake per contraction ( $\mu L./mg. \times 10^4$ )	Contractile tension (Gm./mm. <sup>2</sup> )	Index of mechanical efficiency*
1	1.4	0.85	0.61
4	2.2	2.3	1.04
10	3.33	2.2	0.68
14	2.20	1.3	0.41

\*Index of mechanical efficiency was obtained in this and the next three tables by dividing value in third column by that in second column.

It is postulated in this discussion that the activity oxygen consumption is a measure of the extra oxidative energy required for the synthesis of those high energy phosphate bonds used for contraction, and that the index of mechanical efficiency, obtained as outlined above, is a measure of the efficiency for conversion of the chemical energy available in these bonds into mechanical energy in the form of work. It is also postulated that a comparison of indices of efficiency under different experimental conditions will enable one to determine whether the efficiency of this chemical-mechanical conversion is altered. These postulates are valid only if the 4 following assumptions are valid: (1) that the efficiency of oxidative phosphorylation remains constant; (2) that the resting oxygen consumption which meets the needs of all energy-requiring processes other than contraction is the same for the quiescent and for the stimulated muscle; (3) that oxidative metabolism is essentially the exclusive means of energy production and that glycolysis is insignificant; (4) that the force of isometric contractions is practically proportional to work which would be done in isotonic contraction. It must be admitted that at present there is no experimental evidence available to either prove or disprove the validity of assumptions (1) and (2). On the other hand, the validity of assumption (3) is supported by preliminary work with papillary muscles, as well as by older experiments showing that lactic acid is not produced in beating hearts

Table 6

*The Effect of Frequency of Stimulation on the Mechanical Efficiency of a Cat Papillary Muscle at Constant Resting Tension (4 Gm. per Mm.<sup>2</sup>)*

Frequency of stimulation per min.	Activity $O_2$ uptake per contraction ( $\mu L./mg. \times 10^4$ )	Contractile tension (Gm./mm. <sup>2</sup> )	Index of mechanical efficiency
10	3.20	0.4	0.125
30	2.65	1.3	0.49
90	1.76	2.1	1.19
130	1.18	0.9	0.76

under good aerobic conditions.<sup>26</sup> Finally, recent work in this laboratory, using papillary muscles under isotonic rather than isometric conditions, supports assumption (4) that isometric contractile force is proportional to isotonic work as long as the resting tension on the muscle is the same under both conditions.

A number of examples of comparisons of indices of efficiency under different experimental conditions that lead to marked alterations in force of contraction are shown in tables 5, 6, 7, and 8. These examples, all of which represent experiments on single papillary muscles, come partly from published work of K. S. Lee<sup>25</sup> and partly from unpublished work. In all of the examples given there are marked decreases in efficiency under those experimental conditions associated with decreases in contractile tension.

Tables 5 and 6 show the effects of variations in resting tension at constant frequency of stimulation (60 per minute) and the effects of variations in frequency at constant resting tension. In the experiment at constant frequency, efficiency reaches a maximum at a resting tension of 4 Gm./mm.<sup>2</sup> and then falls off at higher tensions. The finding of an intermediate resting tension at which the index of mechanical efficiency is highest is not too surprising in view of earlier work on the intact heart, which indicated that the ratio of measurable external work to total oxygen consumption is maximal at an intermediate level of left ventricular diastolic volume.<sup>28</sup>

In the experiment at constant resting tension (table 6), the index of mechanical effi-

Table 7

*The Change of Mechanical Efficiency of a Cat Papillary Muscle During Failure In Vitro and after Recovery with Ouabain*

Experimental condition	Relative activity $Q_{02}^*$	Relative contractile tension*	Relative index of mechanical efficiency
Prior to onset of failure	1	1	1
During failure	0.90	0.32	0.35
Recovery from failure with ouabain	0.91	0.71	0.78
(initial therapeutic stage)			

\*Relative rather than absolute values are used in this and the following table.

ciency rises steadily with increasing frequency up to about 90 beats per minute and then falls off as the frequency is further increased. The papillary muscle, like a number of other cardiac preparations including isolated guinea pig and rabbit atria, exhibits an increase in contractile tension with increasing frequency over a fairly wide range of frequencies. This well-known "positive staircase" phenomenon is apparently the result of an increase in mechanical efficiency with increase in frequency and not the result of an increase in utilization of chemical energy. Indeed, on the basis of the activity oxygen consumption per contraction, it appears that the chemical energy utilization per contraction actually decreases as the frequency increases. If the postulate that the index of mechanical efficiency is a direct measure of the efficiency of conversion of phosphate bond energy into mechanical energy is correct, then the marked decrease in contractile strength at low frequencies may be attributed to a very low efficiency for this crucial conversion.

In a previous report, one of us (R.F.F.) suggested that the positive staircase effect in isolated guinea-pig atria was dependent on the rate of some activation process occurring between beats, and that the degree of activation of the cardiac muscle at the time of an action potential determined the size of

Table 8

*The Effect of  $Ca^{++}$  Concentration in the Medium on Mechanical Efficiency of a Cat Papillary Muscle*

$Ca^{++}$ concentration in medium	Relative activity $Q_{02}$	Relative contractile tension	Relative index of mechanical efficiency
No $Ca^{++}$ (early stage)	0.22	0.06	0.27
$Ca^{++} 1.2 \times 10^{-3}$ M	0.71	0.5	0.70
$Ca^{++} 2.4 \times 10^{-3}$ M	1	1	1
$Ca^{++} 7.2 \times 10^{-3}$ M	1.21	1.4	1.16

the contractile response initiated by the action potential.<sup>29</sup> It was proposed that, at low frequencies associated with small contractions, the rate of the activation process was so slow that the degree of activation attained by the time of the next action potential was relatively small; and that, at high frequencies associated with large contractile responses, the rate of the activation process was very much faster, so that the degree of activation at the time of the next action potential was relatively great. In view of the present findings, it appears that this proposed activation process may in reality be a process of restoration of a state in the muscle that determines the efficiency of the conversion of chemical to mechanical energy.

In table 7 are the results of an experiment on spontaneous failure and recovery from failure after addition of a cardiac glycoside. It is apparent that the failure is due primarily to a loss in mechanical efficiency, rather than to a loss in energy production, and that the recovery from failure with ouabain is due to an increase in mechanical efficiency rather than to an increase in energy production. These results, also, are not too surprising in view of older work on intact hearts, which indicated that certain types of failure were associated with a decrease in the ratio of measurable external work done by the heart to total oxygen consumption of the beating heart, and that recovery from failure with cardiac glycosides was associated with an increase in this ratio.<sup>30</sup> However, it was impossible to separate activity oxygen consumption from total oxygen

consumption in these earlier studies, and thus the changes in overall efficiency could not be claimed to demonstrate directly changes in the efficiency of the conversion of chemical to mechanical energy in the contraction process alone. The present findings, on the other hand, do support strongly the concept that there is a loss of efficiency in this conversion in spontaneous failure, and a recovery of efficiency during the restorative action of cardiac glycosides.

Table 8 shows the results of an experiment in which the calcium content of the incubation medium was varied over a wide range. With increases in calcium there is an increase in activity  $Q_{02}$ , contractile tension, and the index of mechanical efficiency. From the data one may conclude that the increase in contractile tension with increase in calcium is due in part to an increase in chemical energy utilization per contraction and in part to an increase in efficiency of the conversion of this energy into mechanical energy. Thus, the reduction of the contractile force brought about by reduction of the extracellular calcium is probably attributable both to a decrease in the utilization of high energy phosphate bonds per contraction and to a decrease in efficiency in the conversion of the chemical energy of those bonds used into mechanical energy.

From the results of experiments such as those discussed in this section we may conclude that in many experimental conditions in which a decrease in contractile force has been attributed to a deficiency in the utilization of high energy phosphates for contraction, the deficiency is in large part, and sometimes almost exclusively, due to a loss in efficiency in the conversion of chemical energy of high energy phosphate bonds into mechanical energy. This conclusion is admittedly based on interpretations, the correctness of which depends on the validity of certain assumptions that have not yet been strictly proved. However, the conclusion presents an interesting working hypothesis, and confronts us directly with the problem of what factors control the efficiency of utilization

of high energy phosphates for mechanical work in heart muscle. Speculation about these factors is beyond the scope of the present paper.

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## Discussion

**Dr. R. E. Davies (Philadelphia, Pa.):** It seems that we and Dr. Mommaerts have been floundering in the same morass for 8 years or so. Concerning the question of whether the creatine phosphate-ATP system changes during a single twitch, he has announced that it does, that it doesn't, and now again that it does. Originally, we found that it did not. Then, about 3 years ago, we found a very labile compound, which we called XP, that was present in resting muscle and not in contracted muscle. After announcing this discovery, we kept on working and found that sometimes it could be seen and sometimes it couldn't. Finally, we decided that XP was a will-o'-the-wisp—or a Wilfried-o'-the-wisp.

We, that is, Drs. Cain, Delluva, Kushmerick, and I then stopped trying to find an unknown compound and tested known compounds to find the source of the inorganic phosphate we had found to be liberated in a single twitch. The content of each compound we tried—however esoteric and unstable—remained unchanged. Finally, despite Wilfried's published results, we determined the creatine content and found that it did change. Then we learned from Wilfried that recently he, too, had had inconsistent results. In seeking the cause for this inconsistency, we examined the dates when the experiments had been done. We found that every time the creatine content hadn't changed, the experiments had been done in spring or summer; and, every time it had changed, the experiments had been done in the fall or winter. Whenever XP was there and changed, it was spring; whenever it either wasn't there or didn't change, it was fall. Perhaps this seasonal variation means that there may be an exceedingly labile compound that can be detected only in the spring or summer; at other seasons it may change and be reconstituted so quickly that we miss it.

Thus we must wait for spring, or go to South America, or start injecting frogs with pituitrin, or perhaps choose an animal that

doesn't have this seasonal variation. Then, maybe in another 2 years, we will find that we are just where we are today.

**Dr. Kwang Soo Lee:** I should like to ask Dr. Mommaerts a question. You mentioned that there is a possibility that heat of activation and heat of shortening and heat of work might come from different metabolic pathways. Is there any experimental evidence that even suggests this?

**Dr. Mommaerts:** Not so far. As I implied, in most of the experiments the only detectable breakdown of phosphocreatine liberates equivalent amounts of phosphate and creatine. There are some circumstances in which a sizable degree of phosphorylation takes place as well, with hexose phosphate formation—less phosphate than creatine is liberated. For a season in which the formation of hexose phosphate was relatively prominent, we plotted either the fraction that goes to phosphate or to hexose phosphate as a function of the total shortening; there was no correlation whatsoever. So far, then, we have had no indication of different chemical reactions serving 1 purpose or the other.

**Dr. Podolsky:** There are some early experiments of A. V. Hill which suggest that there is only 1 driving chemical reaction (*Proc. Roy. Soc. London s. B* 127: 297, 1939). He defined *initial energy* as the sum of the work done and the heat liberated while the muscle is active. The *recovery heat* is the heat liberated while the muscle recovers from activity. The initial energy is liberated quickly compared with the recovery heat, so these quantities are experimentally separable. The *total energy* is initial energy plus recovery heat, that is, the total energy exchange associated with activity.

Hill measured energy exchanges under a variety of conditions of mechanical loading and, under all these conditions, the ratio of initial energy to total energy turned out to be the same. This should be the case if the driving chemical reaction for the contractile mech-

anism were the same under all conditions of loading. Is this clear?

*Dr. Lee:* Yes, but I don't think it really can be considered as evidence because, thermodynamically, whatever it produces, no matter what pathway you take, it is compensated for eventually.

*Dr. Podolsky:* I think it does constitute evidence for the following reason: If there were 2 different reactions, each having different heats of reaction, and if, under certain conditions, 1 of them proceeded to a greater extent than the other, then Hill would not have found regularity in the ratio of initial energy to total energy.

*Dr. Huxley:* May I ask Dr. Podolsky if he can make any estimates of the number of substrate molecules that need to be bound to the actin filament to produce the necessary tension?

*Dr. Podolsky:* I am afraid that I can not provide you with an answer.

*Dr. Huxley:* I was wondering if it would have to be the same sort of number as Morales (Morales and Botts: *Arch. Biochem.* 37: 283, 1952) had to employ in his mechanism in which it was ATP-binding that gave rise to the change on the electrostatic model. Actually, in that model, you need a tremendous amount of ATP and if, in your model, you wipe that amount of ATP off the actin each time, it would imply you have to split that much every time.

*Dr. Podolsky:* The difficulty with a calculation for the "melting" model is that one needs a good theoretical basis for relating the extent of binding to force. Although this can be done in principle, I have not yet found out how to do it.

*Dr. Palade:* You considered 2 possibilities in contraction, Dr. Podolsky—the sliding and the folding of the actin filaments. How extensive should this folding be to account for the contraction, and is the folding of such a magnitude that it should be easily visible in the electron microscope? In other words, is the folding mechanism still compatible with the present morphologic evidence?

*Dr. Podolsky:* The extent of folding, of course, should be the same as the extent of shortening. Now, the way to do the electron-microscope experiments—and some people have tried this—is to quick-freeze the muscle while it is shortening physiologically and then make sections. In both models, the filaments should return to their original length when the muscle relaxes. The question is whether you can catch the folding before the thin filament has had a chance to relax again. Do you see what I mean?

*Dr. Palade:* Yes, but there are micrographs indicating, for example, that the contraction has gone to the extent that the I-bands have disappeared, and the filaments are still more or less of the expected length on the basis of sliding.

*Dr. Podolsky:* I think that should happen at the end of contraction in both cases. The question is, what generates the shortening while the muscle is contracting, not what is the disposition of the filaments after the contraction has ended.

*Dr. Palade:* So, by the time the preparation is connected, the filaments are back to their original length?

*Dr. Podolsky:* Yes, that is a way of getting around it.

*Dr. John Gergely* (Boston, Mass.): Dr. Furchtgott, in connection with this puzzling problem of failure in efficiency, is there any possibility that the lower tensions one obtains under various conditions are due to the block in the excitation coupling, i.e., is there an inefficiency in transmitting the stimulus to the contractile system? Also, in the cases in which efficiency in terms of oxygen consumption is decreased as compared with the development of tension, is one dealing with an uncoupling at the level of generation of phosphatase?

*Dr. Furchtgott:* First, about excitation coupling: It is quite possible that a block or impairment of this little-understood primary process accounts for the decreased contractile force under some experimental conditions. This may be the situation in the case of the negative inotropic action of acetylcholine on atria, since the markedly shortened action po-

tential may not suffice to give full excitation of the atrial cells. Also, the reduction in force at low extracellular  $\text{Ca}^{++}$  concentrations may be due in part to an impaired excitation coupling. However, I would not expect an impairment of this primary process to cause a marked decrease in mechanical efficiency (as indicated by a marked fall in the ratio of mechanical work to activity oxygen consumption) such as we found on reducing frequency or allowing spontaneous failure to occur in papillary muscles.

As for the question of whether an uncoupling at the level of generation of high energy phosphate may account for the decreased efficiency, I think the answer is that such uncoupling of oxidative phosphorylation can probably be ruled out in certain cases of decreased efficiency that we have studied—namely, those involving low tension, low extracellular  $\text{Ca}^{++}$ , and spontaneous failure. In all of these cases, the resting oxygen consumption was about the same or somewhat lower than that under control conditions, whereas a greater resting oxygen consumption would have been expected if there was uncoupling at the level of generation of high energy phosphates.

*Chairman Taggart:* I think Dr. Mommaerts has been waiting to speculate on this.

*Dr. Mommaerts:* Yes, I feel very definitely that it has to do with excitation-contraction coupling, but probably in a way that is not revealed by the duration of the action potential. Dr. A. Y. Brady (unpublished data) has felt that in the heart (not in skeletal muscle) there is a definite correlation between the duration of the action potential and the duration of the active state. It is as if the action potential or its plateau turns the active state on or off.

However, all the inotropic changes that we have encountered in the heart are not primarily caused by a change in the duration of the active state. They are caused by a change in the force-velocity relation, so that, within the same available time, a greater or smaller fraction of the active state is realized in terms of external tension. I don't know whether any

patients are going to be helped directly by this knowledge, but one can say that heart failure is a disease of the constant  $b$  or perhaps of the constant  $a$  in the Hill equation.

*Dr. Hoffman:* Dr. Mommaerts, I wasn't sure if the variation in the constant for the activation was primarily a function of resting length, resting tension, or contractile tension. If the variability is primarily a function of resting length, is it related in any way to the sarcomere length or, let's say, to the extent of overlap of thin and thick filaments in a given sarcomere?

*Dr. Mommaerts:* It may very well vary, Dr. Hoffman, with the resting length, but I also have in mind the variation with the contracting length. Among others, Aubert has studied extensively the maintenance heat as a function of the contracted length (*Le couplage énergétique de la contraction musculaire*. Bruxelles, Editions Arscia, 1956). Over certain ranges, there is a linear relation between maintenance heat and tension. One enters here into a form of philosophy which considers that getting a linear relation is the highest purpose of the scientist. Accordingly, one might believe that it is the tension during activity that has a primary influence upon the maintenance heat.

Of course, tension and length are both changed, and, apart from the linearity, who is to tell which of these factors is of primary importance in determining the maintenance metabolism, or even whether this is a valid question? There is, however, a very real operational difference between the 2 concepts, which appears when evaluating the energy mobilized in a tetanus of constant duration in which a given degree of shortening is allowed. In one instance, with a light load, the shortened length is reached rapidly; in the other, with a heavy load, slowly. Which value of the maintenance heat will be operative? That determined by the varying length, or that determined by the tension? The answer is not available, but the distinction may well be accessible to experimental analysis.

*Dr. Podolsky:* I have had a chance to think about the earlier questions a bit more. In re-

ply to Dr. Huxley, one reason Morales (Morales and Botts: *Arch. Biochem.* 37: 283, 1952) needed so many particles to bind to the polymer is that, in an electrostatic mechanism, the force is reduced by the ionic strength of the milieu, and in muscle the ionic strength is quite high. On the other hand, the polymer melting process need not be a function of ionic strength (although it may), so there is a chance that it might not require nearly so many particles to generate the force. This could be especially true if the polymer had a high degree of crystallinity; in this case, melting is a cooperative process.

About Dr. Palade's question, although there is no published electron microscope evidence that supports folding, there is some physiologic evidence. In both the sartorius muscle (experiments of Buchthal and Kaiser: *Dan. Biol. Medd.* 21: 121, 1951; and of Marechal: *Arch. internat. physiol.* 63, 128, 1955) and in the heart (experiments of Rosenbleuth and Rubio: *Arch. internat. physiol.* 68: 181, 1960), it was found that, if shortening starts from beyond a certain length, the muscle has a memory. The force developed at a shorter length depends on whether that length was reached by active or passive shortening. This

phenomenon is known as hysteresis in the length-tension curve. In the sliding model, the shortening muscle should have no memory but, in the folding model, it could have a memory because there is a point of reference—the point of attachment of the thin filaments upon activation.

In the case of the frog sartorius muscle, there is no memory if the initial length is less than that length at which the H-zone vanishes; that is, if the thin filaments meet at the center of the A-band. However, at these lengths you would not expect it to have a memory because now the ends of the thin filaments would always attach at the same point, the center of the A-band. Although the argument is indirect, this physiologic evidence can be taken to support a folding rather than a sliding model.

*Chairman Taggart:* I am sure that this discussion could go on through the night. It is quite evident, I think, to all of us in the audience, that the mechanochemical features of muscle contraction are not yet thoroughly elucidated. I should like to thank our speakers for their very illuminating discussions and our audience for their attention and participation.

### The Rewards of Scientific Investigation

It is stranger that we are not able to inculcate into the minds of many men the necessity of that distinction of my Lord Bacon's, that there ought to be experiments of *light*, as well as of *fruit*. It is their usual word, *What solid good will come from thence?* They are indeed to be recommended for being so severe exactors of *goodness*. And it were to be wished that they would not only exercise this vigour about *experiments*, but on their *lives* and *actions*, that they would still question with themselves, in all that they do: what *solid good* will come from thence? But they are to know that in so large and so various an *art* as this of *experiments*, there are many degrees of usefulness: some may serve for real and plain *benefit* without much *delight*; some for *teaching* without apparent *profit*, some for *light* now, and for *use* hereafter; some only for *ornament* and *curiosity*. If they will persist in contemning all *experiments*, except those which bring with them immediate *gain* and a present *harvest*, they may as well cavil at the providence of God, that he has not made all the seasons of the year, to be times of *mowing*, *reaping* and *vintage*.—T. Sprat. *The History of The Royal Society of London.* Ed. 3. 1722. Cited by W. M. Bayliss in the preface to *Principles of General Physiology.* Ed. 4. London, Longmans, Green and Co., 1924, p. xvi.

### III. Hibernation in Animals

Chairman: Alfred P. Fishman, M.D.

#### Introduction

By ALFRED P. FISHMAN, M.D.



Figure 1

TO THE interested bystander, the subject of hibernation is a curious mixture of mystery and of science. Part of the mystery stems from the haze of uncertainty that surrounds the idea of suspended animation; part from the exotic creatures that indulge in hibernation. As a prelude to Dr. Lyman's scientific discussion of hibernation in mammals, I should like to remind you of some of the creatures with which he will probably deal.

Every group of vertebrates, except birds, hibernates. But, of all the hibernating mammals, the hedgehog and the dormouse have emerged as the most popular subjects for study in the laboratory.

The hedgehog is the less familiar of the two. It is a mammal of the order Insectivora, ordinarily about 10 inches long. In figure 1 is illustrated a typical hedgehog on the verge of hibernation. It may be seen that its appearance is characterized by a surface of spines and a short tail. Not evident is its poorly developed brain. When startled or threatened, it rolls up into a ball from which spines protrude in all directions.

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In contrast to the hedgehog, the dormouse is a friend of long standing. It is shown in familiar surroundings in figure 2. Not manifest in this illustration is the fact that it is a small, arboreal, squirrel-like rodent, which is more apt to be found in bushes and trees than at tea parties. But, by the close of the summer festivities, when it has gorged itself to extreme obesity, it curls up into a ball and hibernates until Spring. As will be recalled from figure 2, the dormouse may be roused from its torporous state only to lapse back into suspended animation when external stimulation ceases.

It is to subjects such as these that Dr. Lyman has devoted much of his scientific life. Dr. Lyman has won world renown for his fresh observations and ingenious experiments. By training, he is a comparative anatomist who has exploited the special techniques of physiology and biochemistry to unravel some of the mysteries of hibernation in mammals. He will review for us, in the light of his own researches, the present understanding of the biology of hibernation.



Figure 2

## Hibernation in Mammals

By CHARLES P. LYMAN, A.B., M.A., PH.D.

Hibernation and enforced hypothermia in mammals are widely different physiologic states. Prior to hibernation there are various preparations for the hibernating state, including polyglandular endocrine involution, fattening and/or food storage, and changes in the saturation of depot fat in some animals. The actual causes for the onset of hibernation are unknown, for most hibernators can remain active at low environmental temperatures for long periods. Entrance into hibernation is under precise physiologic control, with heart rate, respiratory rate, and oxygen consumption slowing before a decline in body temperature. A reasonably high blood pressure is maintained during this period and in deep hibernation by an increased peripheral resistance produced in part by vasoconstriction. Homeostasis is continued in hibernation, as evidenced by a normal blood pH, a sensitivity to inspired CO<sub>2</sub>, and a response to ambient temperature below 0 C. by increased metabolic rate. At any time during entrance into hibernation or during hibernation the animal may arouse from this condition. Arousal is a coordinated physiologic event in which the anterior of the body is warmed rapidly by shivering and other heat generating mechanisms, while warmed blood is shunted from the posterior by differential vasoconstriction until the anterior reaches nearly 37 C. The tissues and organs of mammals that hibernate are capable of useful function at lower temperatures than the tissues of mammals that do not hibernate, but a hypothermed mammal that can hibernate will die in hypothermia even though it lives longer and at a lower temperature than a mammal that can not hibernate. Hibernation must involve a resetting of the "physiologic thermostat," which thus permits a controlled cooling of the animal, but the nature of this "resetting" is not known.

THE BURGEONING INTEREST in hypothermia for surgery has aroused some curiosity among physicians in the natural hypothermia that occurs seasonally in mammals that hibernate. There can be no doubt that natural hibernation as practiced by many bats, rodents and insectivores is a far less traumatic experience than the hypothermia that is forced on experimental animals, and a study of the former may help to clarify difficulties encountered in the latter. Quite obviously, both hibernation and hypothermia have in common a profound lowering of the usual body temperature of about 37 C., but, beyond this similarity, it is apparent that hibernation in all its phases is a controlled and remarkably regulated

condition, while hypothermia consists virtually in a breakdown of temperature regulation that causes a weakening or collapse of other homeostatic mechanisms.

It has often been stated that mammals which hibernate have an inadequate system of temperature regulation so that, when exposed to cold, their temperatures decline and the animals enter the hibernating state. Actually, however, most hibernators that are not prepared for hibernation can stay active and healthy for months or even years at temperatures that are often fatal to the common laboratory animals of the same size.<sup>1</sup> If, on the other hand, the potential hibernator is prepared for hibernation at the time it is exposed to cold, it may enter the state of hibernation within 24 hours.

### Preparation for Hibernation

The nature of this preparation for hibernation is not clearly understood, but it is certain that the animal must be ready for hibernation or it will not hibernate. Most of the ground squirrel family become extremely

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fat as the season for hibernation approaches, but this does not necessarily seem to be correlated with an abundance of succulent foods. There is some evidence that the fatter animals hibernate before the thin ones, but, fat or thin, they all eventually hibernate during the fall months, and come out of the hibernating phase of their yearly cycle when spring arrives. The length of daily illumination has little, if any, effect upon this cycle, and the evidence to date indicates that the cycle is innate and receives few, if any, clues from the environment.<sup>2</sup>

Unlike the ground squirrels, the golden hamster is not truly cyclic and, if exposed to cold for sufficient time, will enter hibernation at any time of year. Hamsters store large quantities of food prior to hibernation. If denied the ability to store, hibernation is delayed, as if there were some safety check that will not permit the animal to hibernate without adequate supplies for the winter.<sup>3</sup> Hamsters lose fat when exposed to cold prior to hibernation, and the fat that remains is less saturated and hence has a lower melting point than the fat from animals kept in a warm environment (fig. 1\*).<sup>4</sup> This would seem like a nice mechanism to maintain fat in a liquid condition during hibernation, but we have been able to show, in both hamsters and ground squirrels, that hibernation is not delayed when animals are fed a diet that results in depot fat so saturated that it is actually solid in the hibernating animal. Contrariwise, the onset of hibernation is not accelerated if the animals are fed a diet that results in body fat with a low melting point.

In the small rodent hibernators, lack of nourishment may rapidly induce hibernation. The metabolic budget of a diminutive mammal is extreme, for the high surface-to-mass ratio results in a disproportionately large heat loss. When denied metabolic fuel, the North American pocket mouse (*Perognathus*) enters the hibernating state and thus reduces its imme-

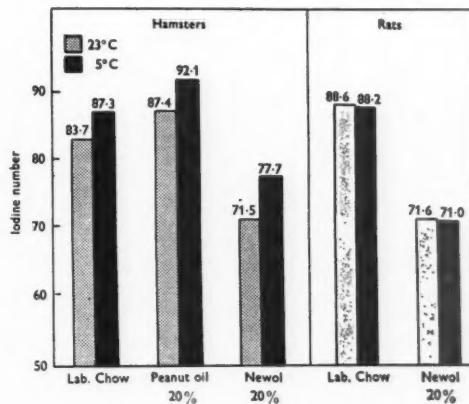


Figure 1

*Effect of cold exposure on the saturation of depot fat of hamsters and rats on various diets. Bar graph on right of each pair is the iodine number of the fat of animals exposed to cold. "Newol" is a highly saturated edible fraction of beef tallow. (From Fawcett and Lyman.<sup>4</sup>)*

diate metabolic problem.<sup>5</sup> This is indeed a hibernation of desperation, for the food supply may be as bad when they awaken as when they entered hibernation. Other small hibernators, notably bats and the European birch-mouse (*Sicista*)<sup>6</sup> usually allow their body temperature to drop any time they become inactive, though on other occasions they remain warm.

Apart from the condition of the animal as far as available food is concerned, there is evidence that the endocrine glands play a role in the preparation for hibernation. Histologic studies have shown that prior to hibernation all the endocrine glands show a marked decrease in activity. Hence it has been postulated that a polyglandular involution must take place before the animal can hibernate.<sup>7</sup> The precise importance of the various endocrines in setting the stage for hibernation has not been elucidated, and some of the observed endocrine involution may be simply incidental. For example, the gonads of most hibernators involute right after the breeding season, which is several months before hibernation occurs. Moreover, it seems reasonably certain that no single endocrine

\*Figure 1 reproduced from Fawcett and Lyman: J. Physiol. 126: 235, 1954.<sup>4</sup> By permission of the Journal of Physiology.

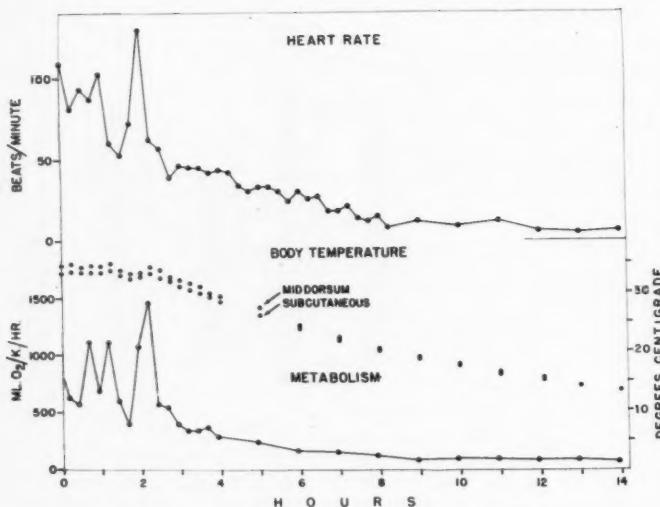


Figure 2

Record of woodchuck entering hibernation, showing chilling and rewarming during the first 2 hours, then a steady decline into hibernation. Heart rate drops first followed by a decrease in oxygen consumption, and then in body temperature. Heart rate and oxygen consumption increase before body temperature when animal rewarms. (From Lyman.<sup>25</sup>)

gland controls the hibernating state, for removal of any one of them does not hasten the onset of hibernation.

#### Hibernation

In spite of its importance in the study of hibernation, the process of entering the hibernating state has been poorly understood. This is because the onset of hibernation is capricious and recording of the various changes involves instruments that can monitor a chronic preparation over long periods. Such instruments were unavailable a decade ago. It is natural to assume that a decline in body temperature would be the first indication of hibernation and that other vital functions would diminish in rate according to the van't Hoff rule as the temperature dropped. Such is not the case, for respiratory rate, heart rate and oxygen consumption are all reduced before a detectable drop in body temperature occurs (fig. 2\*). The contrast between this and enforced hypothermia is striking. In the hibernating animal, hibernation occurs passively as if, as Prosser has so aptly stated, someone had "turned down the thermostat."<sup>28</sup> In enforced hypothermia

the animal chills in spite of a violent metabolic effort to remain warm.

The actual entrance into hibernation is under fairly rigid physiologic control, and the decline in body temperature is always slower than it would be if the "thermostat" were quickly changed from a setting of 37 to 5 C. In most of the hibernators, bouts of shivering, often accompanied by gross muscular movements, take place from time to time. On these occasions the heart speeds and the metabolic rate increases. If the bouts are of long duration, the body temperature ceases to drop and often rises transiently. The result is that the animal may enter hibernation by uneven steps rather than in a smooth curve. At least one hibernator, the California ground squirrel, allows its body temperature to drop only part way toward the deeply hibernating state on its first attempt. With each subsequent entrance, the body temperature drops to a lower level, until it finally reaches a body temperature slightly above the ambient temperature of 5 C. (fig. 3†). It has been suggested that these precisely regulated drops serve to test the state of the animal, so that it never

\*Figure 2 reproduced from Lyman: Am. J. Physiol. 194: 83, 1958.<sup>25</sup> By permission of the American Journal of Physiology.

†Figure 3 reproduced from Strumwasser: Bull. Mus. Comp. Zool. 124: 285, 1960.<sup>29</sup> By permission of the Museum of Comparative Zoology.

lowers its body temperature below a level for which it is physiologically prepared.<sup>9</sup> Furthermore, each entrance into and arousal from hibernation in this animal takes place at a precise time of day, even in the absence of external clues, which suggests that the onset of hibernation is being controlled by some sort of an internal clock.

The circulation during entrance into hibernation in the thirteen-lined ground squirrel is regulated with considerable precision. The first precipitous decline in heart rate, signaling the start of the whole process, causes a drop in blood pressure which, however, remains within the normal values for active animals (fig. 4\*) The heart continues to slow, both by skipping beats and by reduction in the number of even beats (fig. 5). As hibernation deepens, this becomes more and more exaggerated until, in deep hibernation, the heart rate can be as low as 3 beats a minute, with periods of bradycardia lasting 30 seconds or more (fig. 6). Peripheral resistance, as indicated by the slope of the diastolic runoff, increases with the decline in body temperature, so that the mean blood pressure remains at remarkably high levels for such a slow heart rate. Undoubtedly part of the increase in peripheral resistance is due to the increased blood viscosity at low temperatures, but some of it must also be caused by vasoconstriction, for vasodilators and adrenergic blocking agents cause a decrease in peripheral resistance accompanied by an increase in heart rate that may be in part compensatory (fig. 7). Since the temperature in every part of the body declines at an equal rate (see figs. 2 and 4), it is apparent that the vasoconstriction is generalized, and not confined to certain organs or areas.<sup>10</sup>

The preferential ambient temperature for hibernation in most mammals is a few degrees above the freezing point of water. As the body temperature approaches that of the environment, the body temperature curve be-

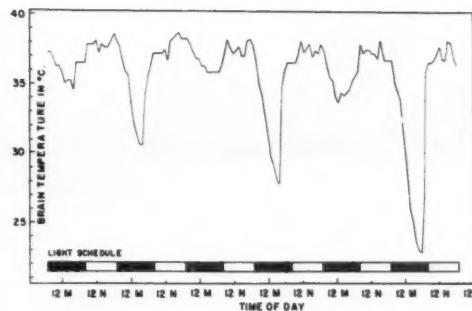


Figure 3

*Brain temperature of a California ground squirrel entering hibernation in the cold during the summer, showing 3 test drops. M and N=midnight and noon. (From Strumwasser.<sup>9</sup>)*

comes asymptotic, and, if the ambient temperature does not suddenly rise, the body temperature always remains slightly higher than the environment. The hibernating mammal is curled in a tight ball, with only the back exposed in the nest, and the low metabolic rate is enough to maintain this temperature difference. Bats, which must roost and hibernate in an extended position, have a body temperature identical with the environment if they are forced to hibernate singly.

In deep hibernation the metabolic rate is often less than one-fiftieth of that of the awake animal at rest, and the body temperature may be as low as 3 C. In spite of this, the animal maintains a remarkable degree of homeostasis. Although the blood sugar is low in some species during hibernation, it is normal in others. The pH is essentially normal, and  $\text{pCO}_2$  is low compared to the active animal. In hibernation the respiratory centers remain remarkably sensitive, for an increase of ambient  $\text{CO}_2$  above 4 per cent will cause an increase in the respiratory rate. The hibernating animal also retains a certain degree of homeothermism. Between ambient temperatures of about 4 and 15 C., the body temperature passively follows the temperature of the environment. If the ambient temperature slowly drops to 0 C. or lower, the metabolic rate is increased and body temperature is maintained above the freezing point. Some-

\*Figures 4, 5, 6, 7, 9 reproduced from Lyman and O'Brien: Bull. Mus. Comp. Zool. 124: 353, 1960.<sup>10</sup> By permission of the Museum of Comparative Zoology.

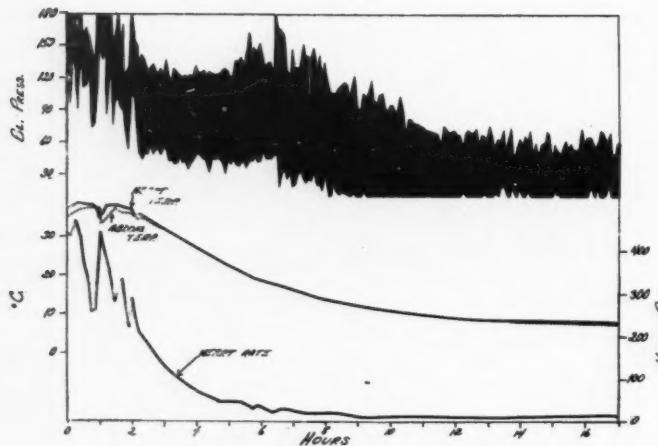


Figure 4

Blood pressure (mm. Hg), heart and abdominal temperature and heart rate of a thirteen-lined ground squirrel entering hibernation. Blood pressure in dark area is highest systole and lowest diastole recorded for a 1-minute period at 5-minute intervals. Heart rate and blood pressure decline before body temperature. Heart remains slightly warmer than abdomen in hibernation. (From Lyman and O'Brien.<sup>10</sup>)

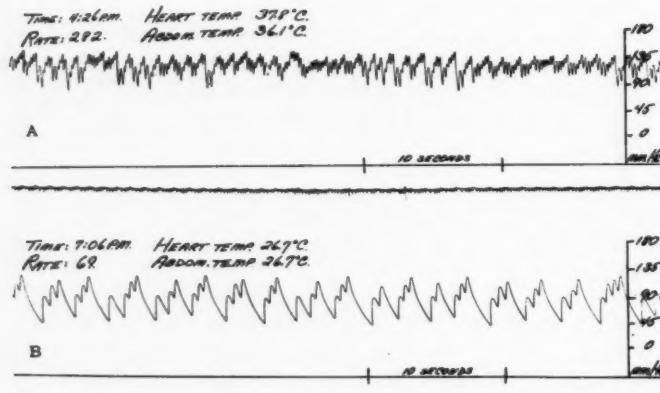


Figure 5

A. Blood pressure and EKG of animal graphed in figure 4. Uneven pattern of beats is typical for this stage. 4:26 p.m. = 1½ hours on figure 4, B. Same animal later. Pattern of beats and skipped beats is now even. (From Lyman and O'Brien.<sup>10</sup>)

times the animal remains in hibernation with a higher metabolic rate, and on other occasions the metabolic effort is sufficient to cause arousal from hibernation. In other cases this rather low-grade temperature regulation is insufficient to protect the animal, and it freezes to death in hibernation.<sup>11</sup>

Deep hibernation does not last throughout the winter in any mammal, for periodically the animal wakes, eats stored food if available, voids, and returns to the hibernating state. The periods of arousal vary with the species. Hamsters generally hibernate for only 3 to 5 consecutive days, ground squirrels for a few days longer, and bats may hibernate for a month or more without waking.

During the periods of hibernation, cell growth and replacement are greatly reduced

but not completely stopped. Although it has been reported that mitotic activity is in abeyance during hibernation,<sup>12</sup> we find mitotic figures in the crypts of Lieberkühn of ground squirrels that have been hibernating continuously for as long as 13 days. Radioactive iron, injected into continuously hibernating hamsters, is found in the erythrocytes, showing that hematopoiesis is continuing, albeit at a very slow rate. The reduced rate of cell production seems to be paralleled by a slow rate of cell aging and destruction, for erythrocytes tagged with chromium remain in the circulation for a much longer time in a hibernating animal than they do in an animal that is active.<sup>13</sup> No experiment has been designed which proves that an animal in hibernation lives longer than its active litter mate,

Figure 6

A. Same animal as figures 4 and 5. Transient increase of heart rate at low body temperature. Note muscle action potentials on EKG denoting shivering.

B. Same animal, now in deep hibernation. Blood pressure slightly damped. Blurring of EKG is electrical artifact. (From Lyman and O'Brien.<sup>10</sup>)

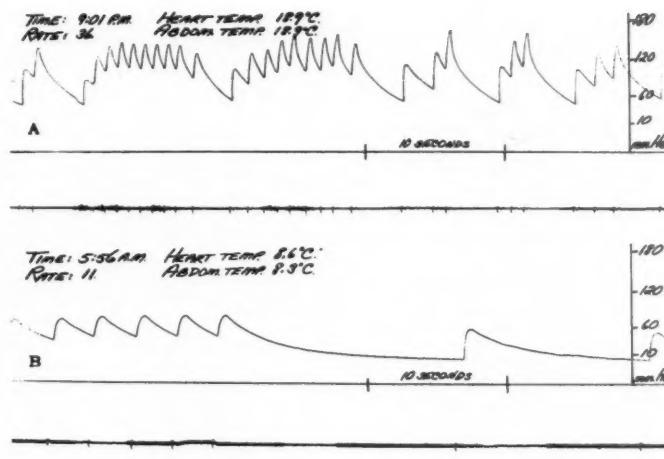


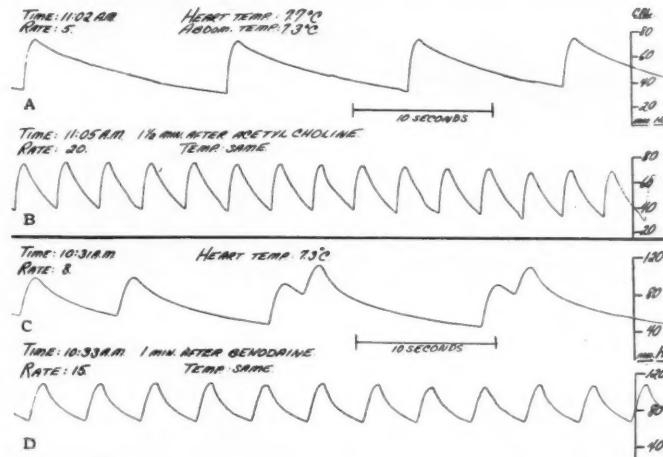
Figure 7

A. Pulse pressure in deep hibernation.

B. Effect of acetylcholine. Faster diastolic run-off from slightly lower systolic pressure than in Figure 7A.

C. Pulse pressure in deep hibernation.

D. Pulse pressure after adrenergic blocking agent. Faster diastolic run-off from same systolic pressure. (From Lyman and O'Brien.<sup>10</sup>)



but it is interesting that bats, which may spend one-half of their life in hibernation, are extremely long-lived animals for their size.

Earlier clinical work had suggested that neoplastic tissue might be differentially killed or its growth slowed by cold, and hibernating animals provided an excellent opportunity to test this premise. Homologous tumors implanted in the cheek pouch of hamsters which then enter hibernation showed no detectable increase in size during the period of hibernation, though on microscopic examination some of the cells appeared to be viable. As soon as the animals awoke from hibernation, however, growth of the tumors

resumed.<sup>14</sup> Heterologous human tumors, implanted in the same manner, also were not destroyed by the 5 C. temperature of hibernation and lived to grow again when the animals awoke.<sup>15</sup>

Animals exposed to radiation during hibernation show little or no cellular damage as long as they remain in the hibernating state. Once they have aroused from hibernation, the cell destruction begins and the length of the animal's life is only prolonged by the number of days it has been in hibernation.<sup>16</sup> The nature of this "radiation memory" is not understood, and its study may be a help in the clarification of the processes involved in radiation injury. Reparative processes, such

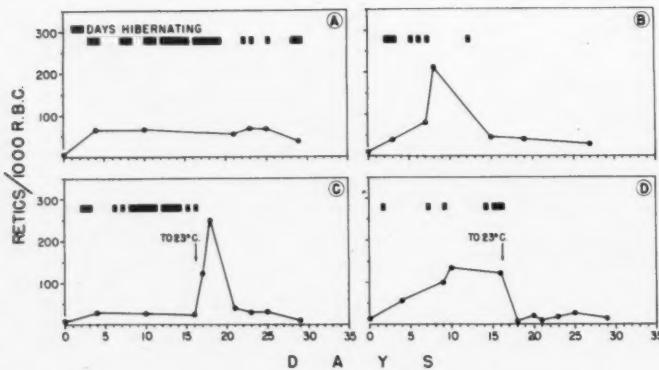


Figure 8

Effect of hibernation on the reticulocyte response of individual hamsters at 5°C. Animals bled on day zero. Hibernation delays and suppresses the response. (From Lyman et al.<sup>17</sup>)

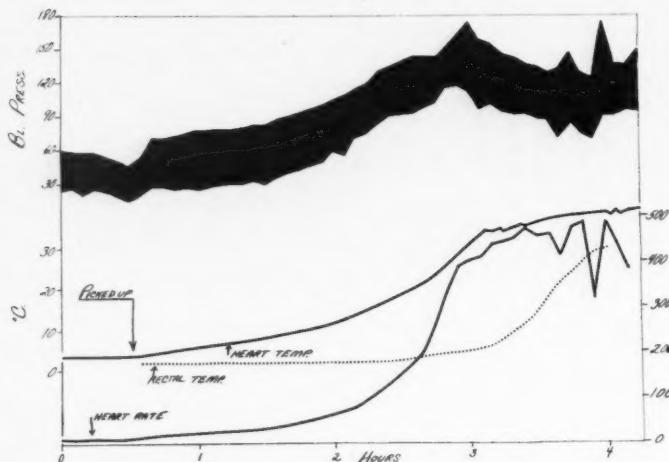


Figure 9

Thirteen-lined ground squirrel waking from hibernation graphed as in Figure 4. Note difference in heart and rectal temperature. Blood pressure drops as rectal temperature rises. (From Lyman and O'Brien.<sup>10</sup>)

as hematopoiesis after bleeding, take place at an extremely slow rate, if at all, during hibernation and it is only after waking that the animal reacts to the stimulus for repair (fig. 8\*).<sup>17</sup>

#### Arousal

Continuous hibernation throughout the winter months would be the most efficient way to avoid an unfavorable situation, but this does not occur in any species that has been studied. Although many theories have been advanced, the cause for periodic arousals is not understood. Arousal may be evoked from an animal in hibernation at any time if a sufficiently strong stimulus is applied, but the strength of the stimulus necessary to

produce the arousal seems to vary with the species and the physiologic state of the animal at the time. The process of arousal is a co-ordinated series of physiologic events in which the hibernator returns to the active state in a minimum of time using only heat generated by its own body. In this orderly sequence, the control of the circulation is remarkably exact, and it is this circulatory control that permits the rewarming with such efficiency. Arousal from hibernation is in sharp contrast to the condition of an animal in enforced hypothermia where the ability to rearm is almost completely lost.

The process of arousal is apparently the same whether the awakening is evoked by an external stimulus or whether it occurs naturally during the hibernating cycle. One of the first signs of awakening is an increase

\*Figure 8 reproduced from Lyman et al.: *J. Exper. Zool.* 136: 471, 1957.<sup>17</sup> By permission of the Journal of Experimental Zoology.



Figure 10

*Radiopaque material injected into heart of normal anesthetized hamster. X-ray taken 2 seconds after start of injection. Note complete circulation of material. (From Lyman and Chatfield.<sup>19</sup>)*

in heart rate accompanied by a decrease in peripheral resistance.<sup>10</sup> The exact sequence of events is difficult to time, but respiratory rate and oxygen consumption rise before a detectable rise in body temperature. In the animals examined to date, the heart rate increases for several minutes before the blood pressure begins to rise (fig. 9). Once arousal is well under way, the rise in blood pressure is rapid and is accompanied by an ever-increasing metabolic rate. Shivering, which at first can only be determined electromyographically, soon becomes so gross that the whole anterior portion of the animal shakes with the effort.

It is characteristic of waking hibernators that the anterior of the animal warms rapidly, while the posterior remains near the temperature of deep hibernation (see fig. 9). This interesting economy of effort is caused by a circulatory control that appears to be an exaggeration of an ability found in non-hibernating mammals. The rat rewarming from hypothermia shows some of the same capabilities but not to such a refined extent.<sup>18</sup> Injection of radiopaque material into the left ventricle of hamsters arousing from hibernation has demonstrated that circulation to

the posterior part of the body is inhibited, and it is reasonable to assume that this is accomplished by differential vasoconstriction so that the circulation is mostly confined to the heart, lungs and brain (figs. 10\* and 11). As arousal continues, the anterior portion of the body warms to nearly 37 C. before the rectal temperature changes markedly. The rectal temperature then rises rapidly and within a few minutes the body temperature of the whole animal has returned to the condition found during the normal active state. While the anterior part of the ground squirrel is warming, the mean aortic blood pressure continues to rise, but, once the posterior starts to warm, the blood pressure either drops or remains level (see fig. 9). It seems probable that the heart is able to develop and maintain a very high blood pressure when the blood flow is restricted but that, when the whole circulation is dilated, it cannot compensate for the decrease in total peripheral resistance. The importance of differential vasoconstriction during the warming process can be demonstrated by intra-arterial

\*Figure 10 reproduced from Lyman and Chatfield: J. Exper. Zool. 114: 491, 1950.<sup>19</sup> By permission of the Journal of Experimental Zoology.

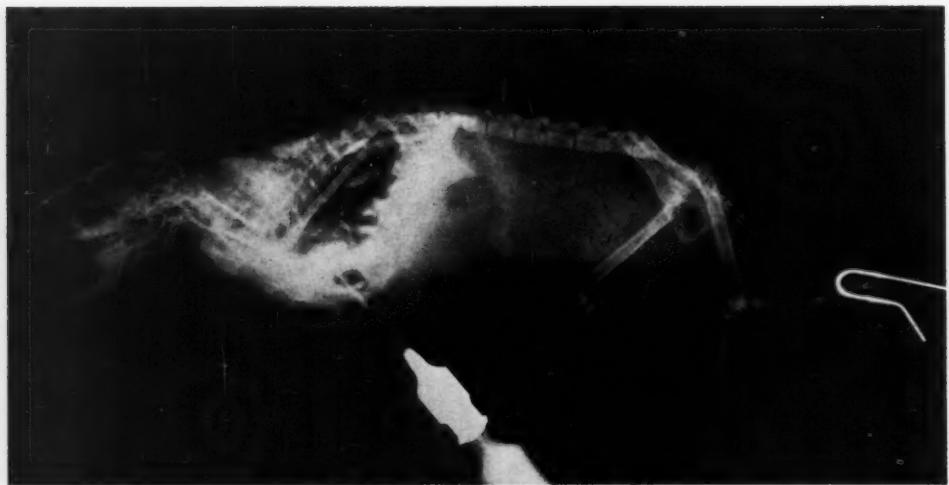


Figure 11

*Radiopaque material injected into heart of hamster waking from hibernation. X-ray taken 4 seconds after injection. Note lack of circulation to the posterior.*

injection of norepinephrine during the waking process. If such an injection is carried out while the rectal temperature is rising rapidly, the temperature ceases to rise for several minutes and the blood pressure increases. A series of such injections at intervals will cause the rectal temperature to rise in a stepwise fashion.<sup>10</sup>

The arousal from hibernation involves a great metabolic effort and it has been calculated that as much energy is spent in one arousal as in 10 days of hibernation. Oxygen consumption rises rapidly as the anterior of the animal warms and reaches a peak at about the time the temperature of the anterior body attains 37 C. At the high point of metabolic effort, oxygen consumption is at least as great as in an active animal under conditions of maximum stress from exercise. The heart rate at the peak of metabolic effort is often 100 times as fast as the slow rate of hibernation. The whole animal seems geared to warm from its low body temperature in the least possible time, and the heart, beating at a rapid rate against a high head of pressure, may be an inefficient pump, but must contribute significantly as a heat source.

If one can generalize about the hibernating cycle by combining the observations on var-

ious species of hibernators, a fairly complete picture may be drawn. The animal in hibernation exerts a minimum metabolic effort conducive to life, deriving energy from stored fat, as evidenced by a respiratory quotient of 0.7. Vasoconstriction maintains a livable mean blood pressure, and other homeostatic mechanisms function efficiently enough to permit existence. However, the hibernating animal is always poised for arousal and even as slight a stimulus as a quick puff of air can sometimes start the waking. Once started, arousal usually is carried to completion. In arousal, the decrease in peripheral resistance is caused by a vasodilation of the anterior portion of the body, while the posterior remains vasoconstricted. Shivering and respiratory movements contribute most of the heat to the waking process, though the totally curarized hamster can warm slowly from hibernation, presumably by using chemically engendered heat.<sup>10</sup> The main source of energy appears to be carbohydrate, for the glycogen of liver and muscle is greatly depleted during arousal. We have suggested that arousal is mediated and driven by a mass discharge from those somatic and sympathetic centers in the central nervous system that control temperature regulation in

the active homeothermic mammal.<sup>20</sup> Although subcortical electrical exploration of the brain of the waking hamster has failed to implicate the hypothalamus in the early part of arousal,<sup>21</sup> there can be no question that the whole process is under precise control and that the autonomic system must play a part in this regulation.

The homeostasis that is typical of hibernation is maintained at body temperatures which are usually lethal to animals that cannot hibernate, and it seems to be typical of hibernators that their organ systems can function at very low temperatures. Thus the peripheral nerve of the Norway rat ceases to function at 9 to 10 C., while that of the hamster will conduct at temperatures as low as 2 C. (fig. 12\*).<sup>22</sup> The hearts of most animals that do not hibernate stop between 16 and 10 C., while the hearts of some animals that hibernate will continue an organized beat at -1 C. At slightly below this temperature, the blood itself would freeze. If the heart rate is plotted against temperature in the perfused, isolated heart of a nonhibernating mammal, the graph is nearly linear, and the temperature at which the heart will stop can be predicted with some accuracy by extrapolating the slope of the temperature-rate curve at high temperatures. Dawe and Morrison<sup>23</sup> were the first to point out that this is not the case in animals that hibernate, for there is a break in the temperature-rate curve at about 15 C., and the hibernator's heart continues to beat at lower temperatures than would be predicted. The hearts of nonhibernating species that are phylogenetically closely related to species that do hibernate show no particular resistance to cold, yet the hearts of all species of hibernators that have been tested to date are resistant to cold even though they may be only remotely related phylogenetically (fig. 13). This suggests that the ability to hibernate may have been developed separately among various species of mammals, and the ability to function at low temperatures is a

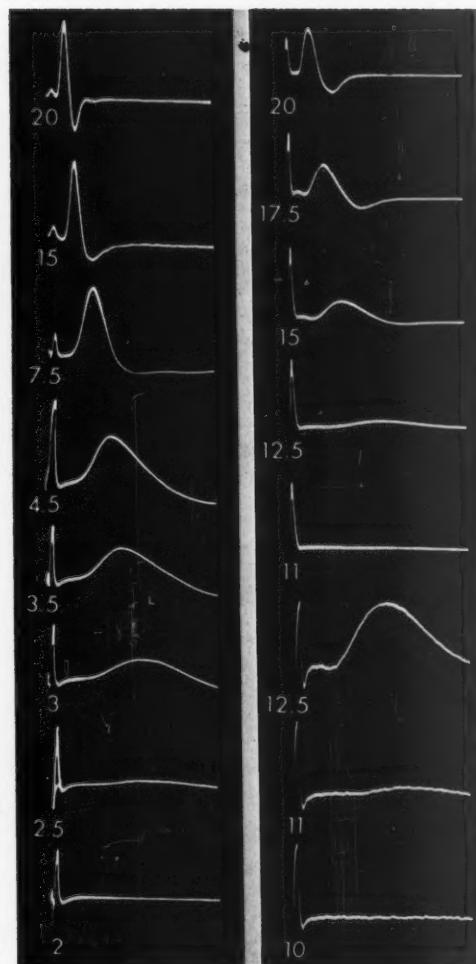


Figure 12

Effect of temperature (C.) on the action potential of the isolated tibial nerve of hamster (left) and rat (right). (From Chatfield et al.<sup>22</sup>)

result of the development of the hibernating habit.<sup>24</sup> For those who consider hibernation a primitive characteristic, it is of some interest that the most primitive living rodent, the mountain beaver (*Aplodontia*), possesses a heart that is as sensitive to low temperatures as one of the most phylogenetically advanced rodents, the Norway rat (see fig. 13).

#### The Physiologic Thermostat

If one grants that the hibernators as a group possess organs and tissues that function

\*Figure 12 reproduced from Chatfield et al.: Am. J. Physiol. 155: 179, 1948.<sup>22</sup> By permission of the American Journal of Physiology.

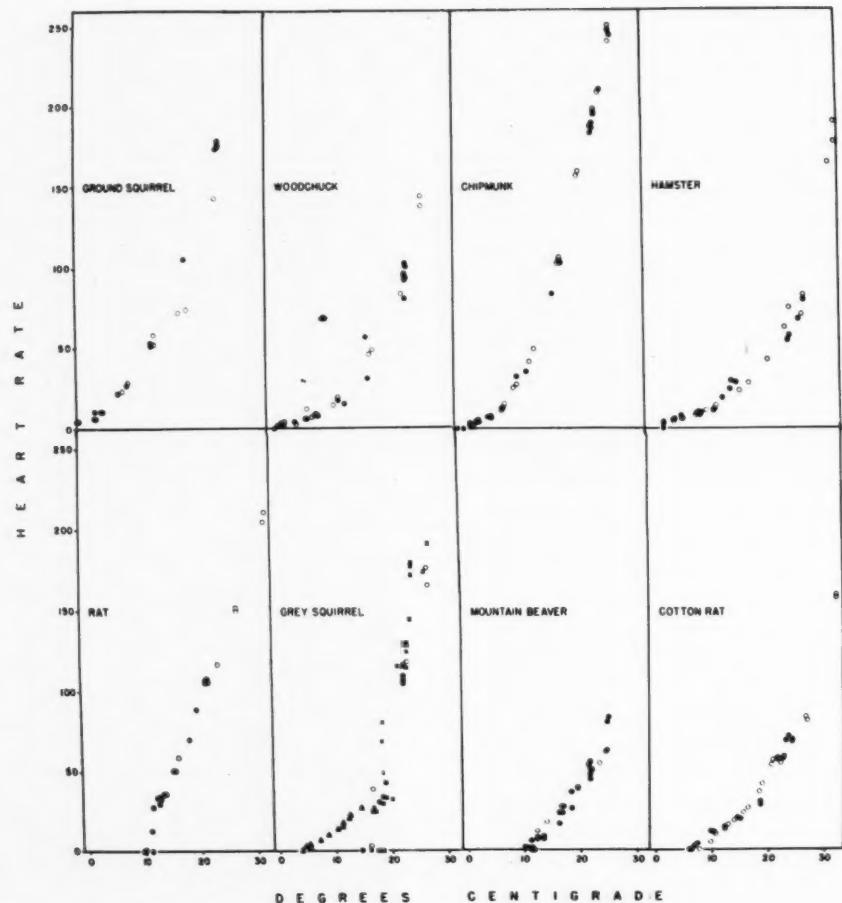


Figure 13

*Effect of temperature on the rate of isolated hearts of hibernators (top four graphs) and non-hibernators (bottom four graphs). Closed circles=first cooling; open circles=rewarming. Triangles on graph of grey squirrel indicate ectopic ventricular beats only.<sup>24</sup>*

at temperatures lethal to nonhibernators, it is not hard to imagine that the hibernating mammal could keep in a steady state by reacting slowly, albeit effectively, to internal or external changes. The problem, however, is not simply one of cells and tissues that can function at unusually low temperatures. When exposed to extreme cold, an active hibernator resists chilling by pilo-erection, shivering, and the other methods of heat generation and conservation that are typical of mammals as a whole. If the cold is overpowering, body temperature drops and the

hibernator enters a hypothermic state. In hypothermia, a hibernator will live longer, and at a lower temperature, than a mammal of the same size that is incapable of hibernation. However, it cannot rearm from near-freezing temperatures without exogenous heat, and if left in hypothermia it will die within 24 hours.

We are thus left with the concept that hibernators have some way of turning down or resetting their "physiologic thermostat." Because of this ability, they have a specialization of temperature control that is unique to them

alone among the vast array of mammals as a Class. The "resetting" must involve changes in both the somatic and autonomic nervous systems, but the precise nature of these changes is largely a mystery. We know that the changes must be coordinated and that every change must be reversible, so that it is not likely that some sort of endogenous metabolic depressant plays a key role in the process. It may be that hibernation is an exaggerated form of sleep. If so, it is a big enough problem to keep us busy for some time.

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## IV. Contractile Proteins

Chairman: Ludwig W. Eichna, M.D.

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### Introduction

By LUDWIG W. EICHNA, M.D.

THIS MORNING'S PROGRAM will inquire into 2 aspects of heart muscle. The first of these is morphogenesis: what is known about the way muscle cells grow and develop, singly, in culture, *in vitro*; and how the various components of the heart develop *in vivo*, accumulate, learn their positions within the organ, and differentiate in the embryo to produce the final structure.

The second aspect will deal with contractile

protein. The function of muscle is to contract, and this unique biologic action is now universally considered to be brought about by the contractile proteins, myosin and actin. The second half of the program will consider these 2 contractile proteins, derived from failing and nonfailing hearts of animals and man. It will consider their biochemical and biophysical properties and their relationship to the genesis of congestive heart failure, particularly as it is seen in man.

Without further ado, I should like to call on Dr. Irwin R. Konigsberg, who will consider "Some Aspects of Myogenesis in Vitro."

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From the Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, New York.

### The Size of the Heart

... it is not absolutely true that people having large hearts are cowards, and on the other hand people having small hearts courageous. Avicenna expresses himself well by saying that in those who have large hearts and are cowards, the size of the heart is out of proportion to themselves and that such people ought to be called true cowards with their large hearts. Thus, when courageous persons have small hearts, they are really courageous. However, there might be courageous persons with hearts of the right and proportioned size.—R. Eriksson. *Andreas Vesalius' First Public Anatomy at Bologna, 1540: An Eyewitness Report*. Uppsala and Stockholm, Almqvist & Wiksell Boktryckeri Ab, 1959, p. 237.

## Some Aspects of Myogenesis in Vitro

By IRWIN R. KONIGSBERG, PH.D.

Suspensions of embryonic chick leg muscle cells have been employed to establish replicate monolayer cultures. Such cultures grow rapidly to form a confluent layer of cells. Despite culture conditions generally assumed to be "dedifferentiative," a high degree of differentiation is attained in terms of the development of cross-striated myofibrils and contractility. To evaluate the possible role of *in situ* nuclear replication in the development of multinuclearity in muscle cells, an inhibitor of deoxyribonucleic acid (DNA) synthesis, methyl-bis (beta-chloroethylamine) (nitrogen mustard), was employed. Treatment with nitrogen mustard at concentration levels that profoundly inhibit DNA synthesis does not block the formation of multinuclear cells. On the basis of the pattern of nuclear enlargement after nitrogen mustard treatment, the cytologic picture of treated cultures is interpreted as indicating that the nuclei of only mononucleated cells are normally capable of proliferation. An absence of proliferative activity in the nuclei of multinuclear cell suggests that myoblast proliferation is self-limiting in this system and may explain, in part, the high degree of differentiation attained in monolayer culture.

**I**N DEVELOPMENTAL BIOLOGY, as in the biologic sciences generally, a significant factor in the development of an area of research is often the choice of the most suitable system. The system should permit ready manipulation of its components, rigid control of environmental factors, and accurate reproducibility. Tissue and organ culture techniques have, in the past, served admirably in approaching these goals.<sup>1</sup> The development of satisfactory techniques for preparing cell suspensions from organized tissues<sup>2, 3</sup> and for cultivating such cells on a glass substratum<sup>4</sup> offers a degree of precision difficult to equal.

Although tissue disaggregation has been applied fruitfully to the problem of cell affinities and the factors controlling the reorganization of tissue architecture,<sup>5-10</sup> the extension of the technique to the cultivation of dispersed cells has not been widely applied to developmental problems.<sup>11-17</sup> This is understandable

in view of the prevailing opinion, which holds that cellular differentiation depends on cell density.<sup>18</sup> With respect to this viewpoint, cell-culture technics may present an opportunity to explore the nature of such a dependence.

The present studies deal with the use of monolayer cultures of cell suspensions to examine cellular differentiation in embryonic muscle cells. Embryonic skeletal muscle cells grow in cell culture and, when confluence is approached, differentiate into elongated multinuclear cells in which the development of typical cross-striated myofibrils occurs progressively and which acquire the ability to contract vigorously.<sup>19</sup>

### Culture Methods

The sparsity of extracellular connective-tissue fibrils and the relatively greater extracellular space of embryonic tissues render them more readily dissociable by brief treatment with dilute trypsin than adult tissues. Leg muscle from 11- to 12-day-old chick embryos can be disaggregated by a 10-minute incubation at 37°C. in 0.05 per cent crude trypsin. Tryptic action is stopped by diluting the incubation mixture with an equal volume of cold complete growth medium. Any undigested tissue residue is removed by successive filtration through gauze and 200-mesh bolting silk. The filtered cell suspension is centrifuged at low speed (1,000 r.p.m.), the supernatant decanted and the cells are resuspended in fresh growth medium. The cell suspension is then counted in an ordinary hemocytometer, and the

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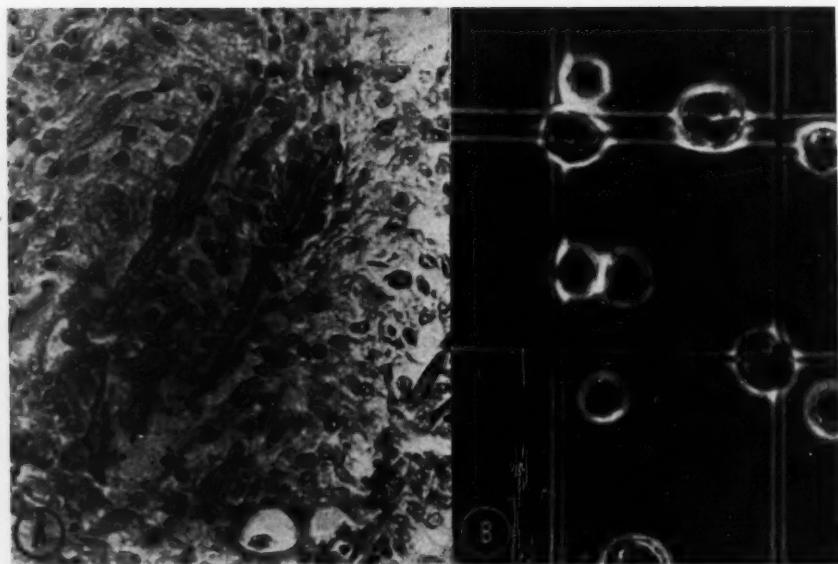


Figure 1

A. Section through leg muscle of 12-day chick embryo fixed in Allen's B15, stained with hematoxylin and eosin. Note the two prominent multinuclear cells in the center of the field. ( $\times 210$ .) B. Cell suspension of embryonic leg muscle cells photographed in the hemocytometer chamber. (Phase contrast.  $\times 500$ .)

desired aliquots are delivered to an appropriate culture vessel—for most purposes, a 5 cm. diameter Petri plate.

#### The Initial Inoculum

One of the more disagreeable aspects of differentiation from the experimenter's viewpoint is its lack of synchrony. Thus, in chick leg muscle at 12 days of incubation, although there are large numbers of undifferentiated, mononucleated cells, many of the cells are further advanced. These comprise cells with varying degrees of multinuclearity, as well as some cells that are obviously cross-striated (fig. 1). Careful examination of the cell suspensions, however, indicates that they consist principally of mononucleated cells. Table 1 gives the distribution of nuclei per cell in 4 suspensions smeared, fixed, and stained immediately after preparation. The fate of the larger cells present in the tissue is obscure. They may be damaged during preparation, filtered out with the undigested residue, or, as has been suggested by Rinaldini,<sup>14</sup> broken

up into viable subunits as a consequence of trypsin treatment. The lability of the multinuclear condition has, in fact, been observed in culture.<sup>20-22</sup>

#### Differentiation

The conditions of cell culture are generally assumed to favor neither differentiation nor the retention of differentiative character. This is apparently not true for embryonic muscle cells using the technics described above. Twenty-four hours after the cell suspension is pipetted into the Petri plates, the cells settle out and attach to and flatten against the glass substratum. The culture presents the appearance of a typical culture of "fibroblast-like" cells. Although the cells increase in number, they remain "fibroblast-like" until confluence is approached. At this time, large numbers of extremely long multinuclear ribbon-like cells appear, which crisscross throughout the culture (fig. 2). Using an initial inoculum of  $5 \times 10^6$  cells per 5 cm. Petri plate, this process of multinuclear cell for-

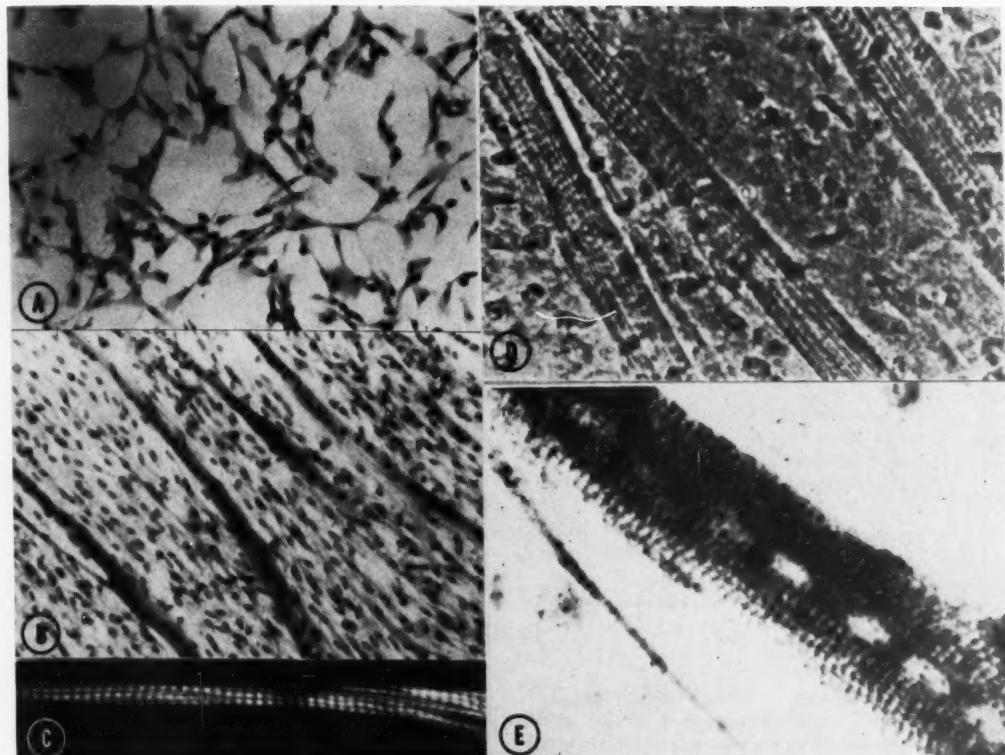


Figure 2

*Developmental sequence in monolayer cultures of embryonic chick muscle cells. A. Cultured cells 2 days after plating present typical "fibroblast-like" appearance (Calcium-formol fixation, Ehrlich's hematoxylin,  $\times 63$ .) B. Multinuclear "ribbon-like" cells that have formed in a sister culture (of A) incubated for 2 additional days. Note the increased cytoplasmic basophilia of the multinuclear cells and the degree of crowding of their nuclei. (Fixation, staining, and magnification as in A.) C. Cross-striated myofibrils in a multinucleated muscle cell in 7-day-old culture. (Polarizing optics,  $\times 400$ .) D. Myofibrillar pattern in a glycerol-extracted 10-day-old culture. (Phase contrast optics,  $\times 400$ .) E. Distribution of reduced tetrazolium (Nitro BT), using DPN-H as a substrate, in a multinuclear muscle cell of an 8-day culture. The regular pattern of deposition mimics the localization of mitochondria adjacent to the I-bands. Note the central row of nuclei visualized by the absence of diformazan granules. (Fresh tissue, 15-min. histochemical incubation, postfixation in cold calcium formol,  $\times 400$ .)*

mation occurs between the third and fourth day of culture. In addition to their multinuclearity, 2 other cytologic features distinguish the primitive muscle cells from the mononucleated cells in culture at this time:

1. The cytoplasm of the multinuclear cell is extremely basophilic. Similar increases in basophilia of myotubes (multinuclear muscle cells with centrally located nuclei, often con-

tiguous) have been observed in vivo and demonstrated to be due to the accumulation of ribonucleic acid.<sup>23, 24</sup>

2. The presence of large numbers of mitochondria in the cytoplasm of the multinuclear cell<sup>25</sup> can be demonstrated histochemically by tetrazolium reduction using either succinate,<sup>26</sup> DPN-H, or TPN-H as a substrate. Although reduced tetrazolium can be localized in mono-

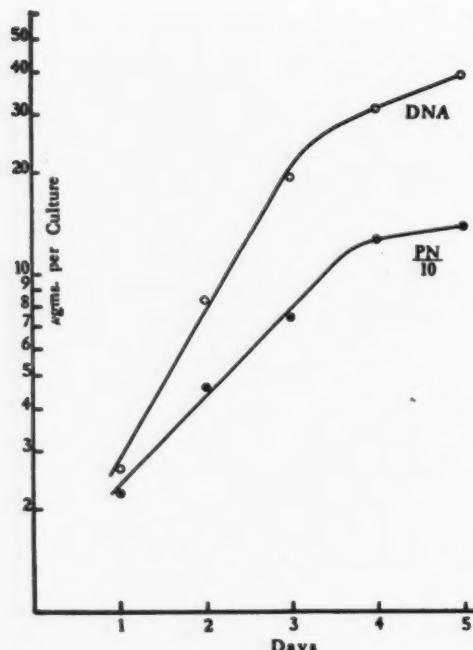


Figure 3

Semilogarithmic plot of the accumulation of deoxyribonucleic acid (DNA), open circles, and protein nitrogen (PN), closed circles, per culture. PN values are divided by a constant factor (10) to permit visual comparison of slopes.

nucleated cells, it is extremely sparse except in the macrophages.

Sometime between the seventh and tenth day of culture, spontaneous contractions of the multinuclear cells can be observed. Spontaneous contraction of skeletal muscle cells in culture was first described by Lewis.<sup>27</sup>

Although cross-striation is not strikingly apparent using routine hematoxylin staining or phase-contrast microscopy, the pattern can be clearly observed with polarizing optics or under phase-contrast microscopy after extraction of the cultures with cold 50 per cent glycerol. Glycerol extraction permits detection of cross-striations between the sixth to seventh day of culture. Although at first the pattern can be observed in only short lengths of a few cells, with time the extent of the pattern and the number of cells in which it can be observed increase. Under polarized

Table 1  
Distribution of Nuclei per Cell in Cell Suspensions Prepared from 12-Day Embryonic Chick Leg Muscle

	Number of nuclei per cell				Total—all classes
	One	Two	Three	Four	
172	24	4	1	1	201
92	11	1	0	0	104
131	32	4	1	1	168
181	35	2	1	1	219
Totals	576	102	11	3	692
%	83.2	14.7	1.6	0.4	

light, longitudinal birefringent fibrils can be detected even before cross-striation can be observed. Similar fibrils are observed in hematoxylin-stained preparations. As Holtzer has pointed out, it is difficult to know whether such fibrils are in fact unstriated or have a striated pattern below the limits of resolution of the technics employed.<sup>28</sup>

The cross-striated pattern is demonstrable by still a third technic. The mitochondria of striated muscle are arranged adjacent to a particular band (I-band in skeletal muscle, A-band in cardiac muscle).<sup>29</sup> When histochemical tests for either DPN-H- or TPN-H-cytochrome c reductases are applied, the di-formazan deposits are found adjacent to the I-bands as with adult muscle,<sup>29,30</sup> registering the pattern of cross-striations by the localization of mitochondria (see fig. 2).

The progressive nature and degree of differentiation observed in these cultures indicate that dispersed cell culturing, *per se*, is not necessarily incompatible with differentiation. When such incompatibility is observed, any of several possible factors may be responsible. Among these are: (1) enhanced diffusion coupled with an inadequate medium, (2) unrestricted proliferation that might lead to dilution of a developmentally significant cellular component, (3) karyotypic changes, and (4) overgrowth by an altered cell or one of different competence (i.e., fibroblast).

Excessive cell proliferation, whatever the ultimate mechanism, has frequently been implicated in the loss, either permanent or transitory, of differentiative expression. An as-

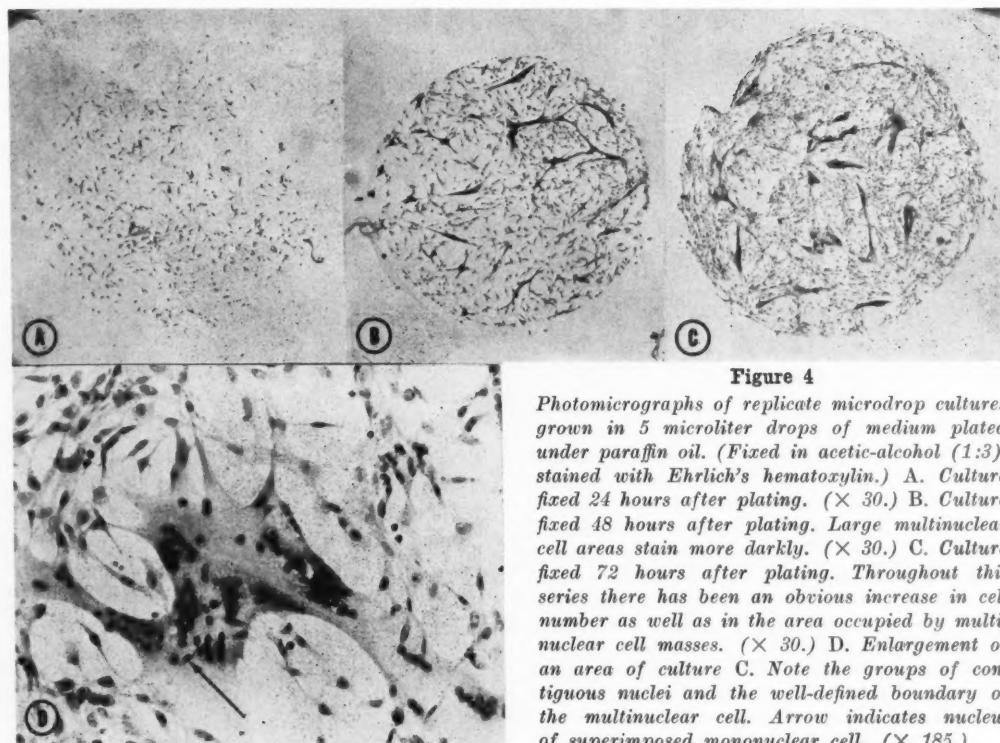


Figure 4

Photomicrographs of replicate microdrop cultures grown in 5 microliter drops of medium plated under paraffin oil. (Fixed in acetic-alcohol (1:3), stained with Ehrlich's hematoxylin.) A. Culture fixed 24 hours after plating. (X 30.) B. Culture fixed 48 hours after plating. Large multinuclear cell areas stain more darkly. (X 30.) C. Culture fixed 72 hours after plating. Throughout this series there has been an obvious increase in cell number as well as in the area occupied by multinuclear cell masses. (X 30.) D. Enlargement of an area of culture C. Note the groups of contiguous nuclei and the well-defined boundary of the multinuclear cell. Arrow indicates nucleus of superimposed mononuclear cell. (X 185.)

sumed antagonism between proliferation and differentiation has long received theoretical attention, at least, from developmental biologists. Indeed, one of the characteristics of organ culture that is commonly employed to study differentiation is reduced proliferation.

An examination of the parameters of culture growth was undertaken to evaluate the possibility of such an antagonism in the system under examination.

#### Biochemical Parameters of Culture Growth

Overall proliferation has been measured by the daily increment of DNA per culture. As an index of cell size as well as cell number, the protein nitrogen (PN) content of cultures has been followed. The relative growth rate\* of DNA (0.0416) agrees well with the maximum rate reported by Harris<sup>31</sup> for the increase in cell number of embryonic skeletal

muscle cells despite the differences in media and technics used. Figure 3 is a semilogarithmic plot of DNA and PN changes per culture for the first 5 days of the culture period starting from an inoculum of  $5 \times 10^5$  cells. Logarithmic increase of both DNA and PN occurs for the first 3 days; PN accumulates during this period at a slower rate than DNA, the relative growth rates being 0.0416 for DNA and 0.0248 for PN. Whether the resultant decrease in PN per DNA indicates a progressive decrease in cell size or differential growth of the various cell types in the culture population is not known.

With an initial inoculum of  $5 \times 10^5$  cells, a break occurs in the rate of accumulation of both DNA and PN between the third and fourth day of culture. The appearance of multinuclear "ribbons" immediately precedes this break. After the break, DNA and PN continue to accumulate but at a much slower rate.

$$k = \frac{2.303 (\log N_2 - \log N_1)}{T_2 - T_1}$$

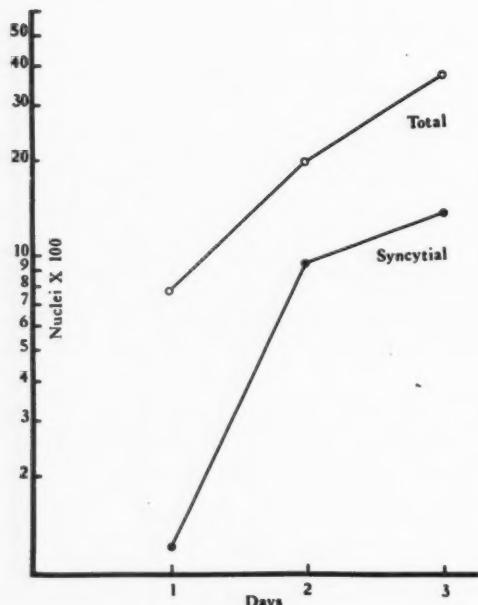


Figure 5

Semilogarithmic plot of nuclear number with time in the total population, open circle, and in syncytial masses, closed circle.

These results extend the qualitative observation that an increase in cell number occurs during the initial period of cultivation. They indicate, moreover, that during this period proliferation is rapid, the population doubling every 24 hours. Although a break in the rate of accumulation of both DNA and PN is temporally associated with the period of multinuclear cell formation, any attempt, at present, to assess the extent to which these events are causally related would be premature.

These data are an indication of overall growth; however, they do not, of course, permit a distinction between the contributions made by the various cell types in heterologous populations such as these.

#### Nuclear Counts

Nuclear counts were made in order to compare the rate of development of multinuclearity with the rate of cell proliferation. Reduction of the population size to magnitudes that would permit counting of nuclei was achieved

by depositing measured microprecipitates ( $5 \mu\text{L}$  volumes) of cell suspension on coverslips submerged under paraffin oil. Paraffin oil has been employed successfully in microprecipitation to prevent evaporation of the medium while permitting gas exchange.<sup>32</sup>

At 24-hour intervals after depositing the microprecipitates, cultures were fixed, stained, and counted. Figure 4 consists of photomicrographs of such cultures fixed at 24, 48, and 72 hours after plating. Grossly it is apparent that the population has increased throughout the period and that the area occupied by multinuclear cells (dark-staining cytoplasm) has increased, at least between the first and second day. Identification of syncytial nuclei is facilitated by the fact that the majority of the nuclei within syncytia are, at this stage, contiguous. Additional criteria are the marked basophilia of the cytoplasm of the multinuclear cells and the more regular spherical shape of syncytial nuclei. The occasional superimposition of a mononucleated cell over a multinuclear cell can be readily detected by differences in focal plane as well as by tracing cell boundaries (see arrow, fig. 4).

In figure 5 values for syncytial and total nuclear counts are plotted semilogarithmically. Both sets of data have been plotted similarly for purposes of comparison. The assumption is made that the increase in nuclear number is an exponential function, an assumption probably applicable to mononucleated cells on theoretical grounds but of perhaps dubious validity with respect to multinucleated cells.

The relative growth rate of the total nuclear population (0.0393) is in good agreement with the relative rate of DNA accumulation (0.0416). The growth rate of syncytial nuclei, however, is twice ( $2.2 \times$ ) the rate of increase of the total population. If the assumption were made that multinuclear cells represent a discrete and separate population of cells in which increase in nuclear number is the result of nuclear replication in situ, the difference in rates of increase of the 2 nuclear populations would become greater still.

If we consider increases in mononucleated

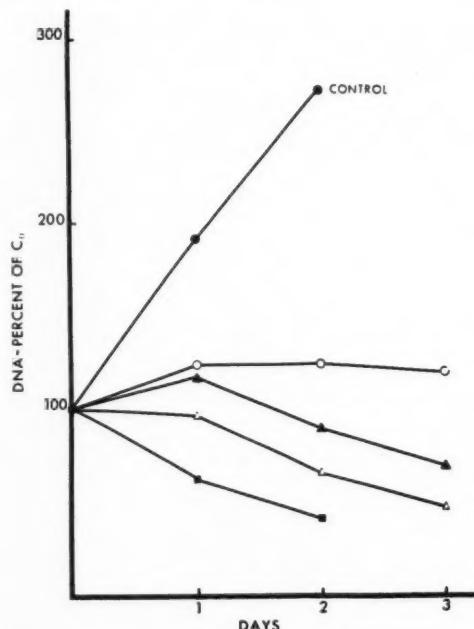


Figure 6

Deoxyribonucleic acid per culture, expressed as percentages of the control level ( $C_0$ ) at the time of treatment with nitrogen mustard. Control, closed circle;  $0.52 \times 10^{-7}$  M, open circle;  $1.56 \times 10^{-7}$  M, closed triangle;  $2.6 \times 10^{-7}$  M, open triangle;  $5.2 \times 10^{-7}$  M, closed square.

cells alone, a relative growth rate of 0.0197 is found, which is less than one-fifth of the rate of increase in syncytial nuclei.

Nuclear counts demonstrate, in addition, that at some stage nuclear division has occurred in the population of prospective myogenic cells. Figure 5 indicates that by the third day of microdrop culture the number of syncytial nuclei is almost twice the total number of nuclei present at 24 hours. Even assuming that all of the nuclei present at 24 hours are of myogenic cells, an assumption not supported by the multiplicity of cell types observed in culture, this is a clear indication of proliferative activity on the part of the myogenic elements.

On the evidence currently available, it seems most likely that this proliferation occurred before the myogenic cells became multinuclear.

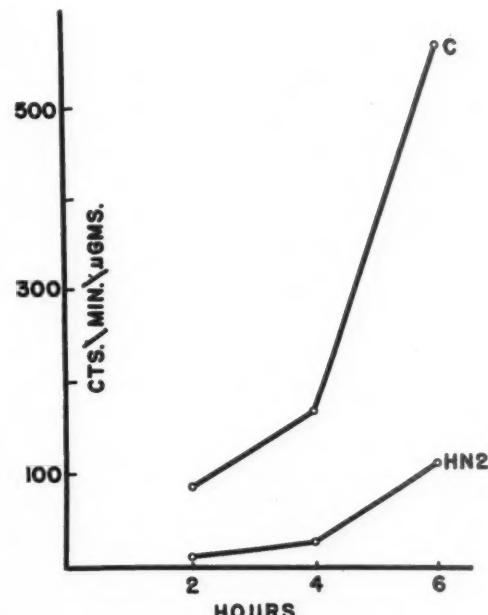


Figure 7

Specific activity of DNA-thymine in control (C) and nitrogen-mustard-treated ( $1.56 \times 10^{-7}$  M) cultures (HN2) during the period from 20 to 26 hours after treatment.

Mitotic figures are rarely, if ever, observed in multinuclear muscle cells.<sup>1</sup> In the extensive cultured material used in these studies they have never been observed. This fact has led to the postulation of 2 alternative mechanisms to explain the development of multinuclearity. One view holds that nuclear replication does occur but by a process of direct splitting of the nuclei. Support for this hypothesis is based largely upon the interpretation placed on cytologic preparations. Various deformations of syncytial nuclei have been described that suggest incipient or recent splitting.<sup>33-35</sup> Alternatively, it has been postulated that multinuclearity results from successive fusion of mononucleated cells.<sup>23, 25, 36, 37</sup>

#### The Dissociation of DNA Synthesis from the Development of Multinuclearity

If multinuclearity is a product of nuclear replication, it would be reasonable to assume the involvement of DNA synthesis. If division

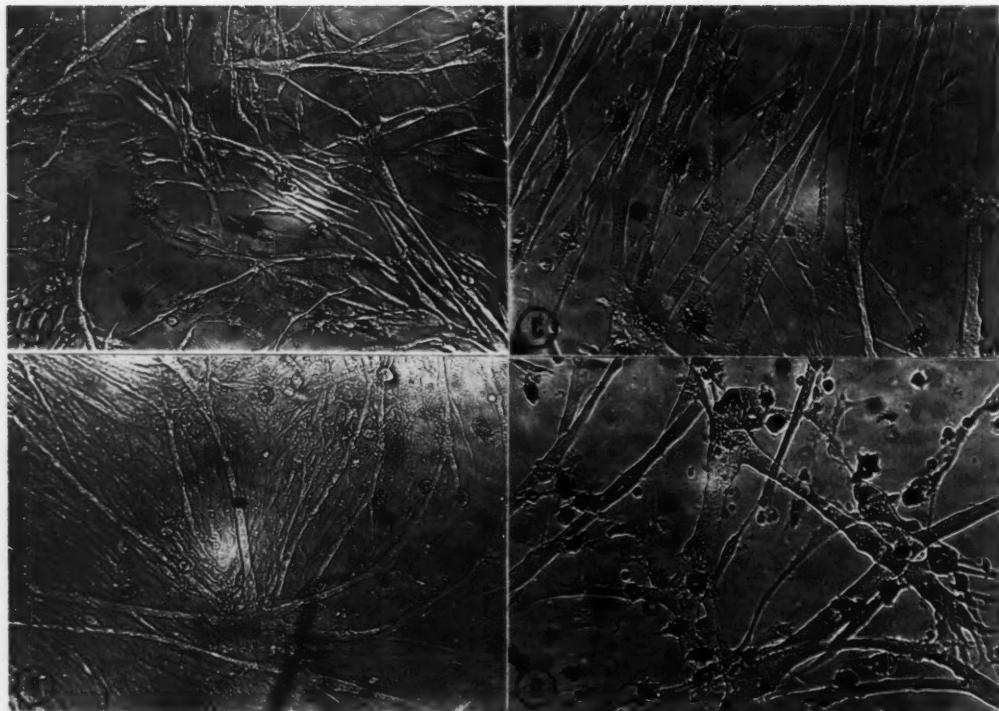


Figure 8

Photomicrographs of living cultures on 3 successive days following treatment with the highest concentration ( $5.2 \times 10^{-7} M$ ) of nitrogen mustard investigated. A. Control culture at the time of application of nitrogen mustard to the experimental group. Multinuclear cell formation has been initiated (note relatively small multinuclear cell). B. Culture 24 hours after treatment with nitrogen mustard. Multinuclear cell formation has continued producing typical elongated broad ribbon-like cells. C. Mustard-treated culture 48 hours after treatment. By this time a marked decrease in the number of mononucleated cells has occurred. No such loss of mononucleated cells occurs in control cultures. Note dark, presumably necrotic, crenated cells. D. Mustard-treated culture 72 hours after treatment. The culture is primarily a network of multinuclear cells with bare interstices normally occupied by mononucleated cells.

occurred without DNA synthesis, the distribution of values for DNA per nucleus within muscle cells should be weighted toward values lower than the diploid value. However, the data of Lash et al. indicate that no such skewness exists.<sup>23</sup>

To test independently the premise that multinuclearity is a product of nuclear replication, an inhibitor of DNA synthesis, nitrogen mustard [methyl-bis(beta-chlorethyl)amine], was employed.<sup>15</sup> Solutions of nitrogen mustard were applied to cultures prior to mas-

sive formation of multinuclear cells. The effects of the inhibitor on DNA accumulation and the incorporation of  $C^{14}$ -formate into DNA thymine are graphed in figures 6 and 7 respectively. DNA accumulation is profoundly affected even at the lowest dosage tested, and the rate of incorporation into DNA thymine is reduced to less than 15 per cent of the control level by this treatment.

Despite these profound effects on DNA synthesis, treatment with even the highest concentration of nitrogen mustard used ( $5.2 \times 10^{-7} M$ )

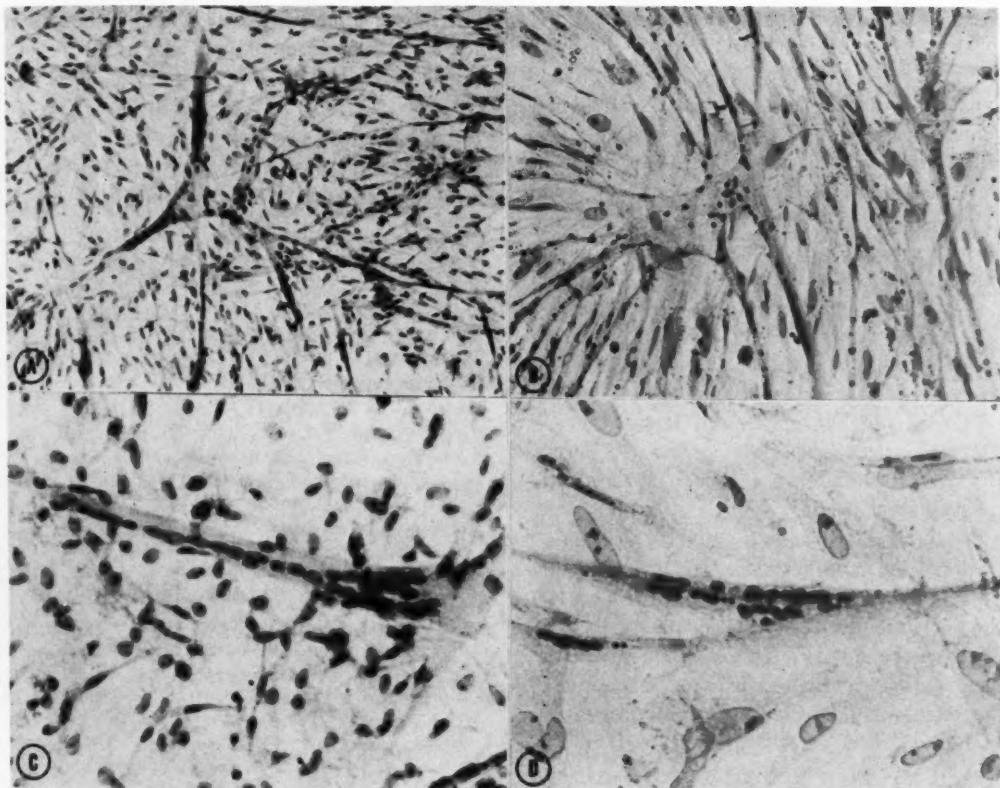


Figure 9

Control and nitrogen-mustard-treated cultures of cell suspensions concentrated and replated, 24 hours after treatment ( $1.56 \times 10^{-7}$  M). Cultures were fixed in 10 per cent neutral formalin and stained with Harris hematoxylin 24 hours after replating. A. Control culture in which multinuclear cell formation has occurred. ( $\times 210$ .) B. Culture of nitrogen-mustard-treated cells. Despite treatment multinuclear cell formation has occurred (linear arrangement of nuclei in more basophilic cytoplasm). The giant nuclei of treated mononuclear cells can be seen between the multinuclear cells whose nuclei are closer in size to the nuclei of control cultures. ( $\times 210$ .) C. Control culture at higher magnification. Note the relatively uniform size of the plural nuclei in the multinuclear cell. ( $\times 495$ .) D. Multinuclear cell of treated culture at higher magnification. Nuclear size is more variable than in the control multinuclear cell (C). Arrow indicates the presence of nuclear fragments or micronuclei. ( $\times 495$ .)

$10^{-7}$  M) did not inhibit the development of multinuclearity (fig. 8). Grossly, there did not appear to be any fewer multinuclear "ribbons" in nitrogen-mustard-treated cultures. The mediation of any lag in the effect of nitrogen mustard was ruled out by testing the ability of treated cells to form multinuclear myotubes during the second day after

treatment. In these experiments the attainment of multinuclearity during the 24-hour incubation period after mustard treatment was circumvented by using sparsely seeded cultures. To test the capacity to form multinuclear cells, these cultures were then trypsinized, the cell suspension concentrated, and denser cultures set up in smaller vessels.

Again, nitrogen-mustard treatment did not prevent the development of multinuclearity (fig. 9). Cytologically the nitrogen-mustard-treated cultures present further evidence which suggests that the nuclei of the multinuclear cells are normally nonproliferative.

In his pioneering studies on the developmental effects of nitrogen mustard, Bodenstein demonstrated that extreme nuclear enlargement and giant-cell formation were commonly observed after mustard treatment.<sup>38, 39</sup> The effects occurred only in those cells that would normally have been described as proliferative. This was most clear-cut in the case of the eye of the amphibian larva where a sharp demarcation exists between the proliferative zone and the zone of postmitotic differentiating cells.

A similar situation exists in nitrogen-mustard-treated muscle cultures. Here the cells that exhibit marked nuclear enlargement are the mononucleated "fibroblast-like" cells. The nuclei of the multinuclear cells that form after treatment are closer to normal size. However, the effect of nitrogen-mustard treatment is evident in these nuclei also by virtue of the wider variation in nuclear size than is normally seen (compare figs. 9C and 9D) and the presence in both cell types of nuclear fragments or micronuclei.

The analogy suggested is that the "fibroblast-like" cells correspond to the cells of the proliferative zone in Bodenstein's work, while the multinuclear cells correspond to the postmitotic differentiating cells.

The results of the studies with nitrogen mustard indicate that nuclear proliferation plays no significant role in the development of multinuclearity in the system under study and are compatible with the conclusions drawn from studies representing a variety of approaches.

Thus, microspectrophotometric analysis of regenerating mouse muscle indicates an essentially unimodal (diploid) distribution curve of DNA per nucleus in regenerating myotubes.<sup>23</sup> Myoblast fusion has been observed<sup>40</sup> and recorded microcinematographically,<sup>22, 41</sup>

It has also been reported that interordinal chimera form in mixed cultures of mouse and chick myoblasts.<sup>42</sup>

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## Differentiation of the Atrioventricular Conducting System of the Heart

By ROBERT L. DEHAAN, PH.D.

The structure and function of the atrioventricular conducting system of the heart, and its relationship to the myocardium, are examined from a developmental point of view. On the basis of information derived from electron micrographie, electrophysiologic, and developmental studies of heart tissue, it is concluded that: (1) The idea of the syncytial nature of the heart lacks a sound anatomic basis. (2) Cytodifferentiation during embryonic cardiogenesis results in the development of at least 2 distinct populations of cells: those comprising the bulk of the myocardium and a second type, the specialized cells of the conductive tissue, which differs in histology, biochemistry, and physiology. (3) The common view of the myocardium as a spontaneously active tissue may require revision, since several lines of evidence appear to indicate that myocardial cells are quiescent until stimulated by an extrinsic source. Under normal circumstances, this stimulus source is the conductive tissue.

THE DEVELOPMENTAL physiologist is interested, not only in the changing functions and interrelationships of organs and tissues in the embryo, he is also concerned with the application of information and concepts obtained from the fields of developmental biology to problems of adult physiology. The problem of the structure and function of the atrioventricular conducting system of the heart is particularly interesting when examined from a developmental point of view.

In the early embryonic heart, the endocardium and epicardium are separated by a thick gelatinous layer, the "cardiac jelly."<sup>1</sup> Distributed through this matrix are mesenchymal cardiac myoblasts, from which the bulk of the myocardium and, presumably, the conductive tissue will form. As the myoblasts begin to differentiate, taking on the characteristics typical of heart muscle, the conducting tissue becomes progressively more easily distinguishable. This divergence may be interpreted in two ways. One is that it results from the simultaneous development of the conductive system and myocardium along dissimilar paths of differentiation. It is often stated, however, that conductive tissue is only "specialized" myocardium in which certain properties, for example conductivity and spontaneity, are exaggerated.<sup>2</sup> Authors tak-

ing this position usually note that these properties are "embryonic" in character. Thus, the second possibility is implied: that the two tissues are basically similar during embryogenesis, differentiating along the same route, but at different rates. At any given stage of development, the conductive system should then be less highly differentiated than the surrounding myocardium, but should not exhibit any qualitatively different characteristics.

We find ourselves concerned, then, with the degree of difference between myocardium and conductive tissue. Are fibers of these two types characterized by distinct histologic, cytologic, and biochemical properties? Do such differences underlie clear-cut physiologic differences of more than a quantitative nature? Do myocardial and conductive fibers, in fact, constitute two different tissues?

I shall examine these questions by drawing upon the literature concerning the physiology and development of the heart of various mammals, and of the chick embryo. Numerous investigators have noted the basic similarities in cardiac development of these forms.

### Histodifferentiation

During the early period of cardiogenesis, curvature and differential growth change the heart from a primitive straight tube to a complex S-shaped organ in which the four adult chambers are clearly represented (fig.

From the Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland.

1). The beat is initiated at the posterior end of the heart. As the primordial atrioventricular (A-V), atrial, and sinoatrial (S-A) regions progressively form, each of them, in turn, takes over the pacemaker function, initiating the beat for the entire heart. At these early stages the primordial atrial and ventricular muscle are in direct continuity around the entire circumference of the A-V canal. A stimulus arising in the sinoatrial region should be able to spread throughout the heart, freely to all areas. That this is true has been demonstrated by Patten<sup>3</sup> who cut away all of the tissue around the A-V canal of a 4-day chick heart except for a narrow connecting strand (fig. 2).<sup>\*</sup> This strand then served as an "artificial bundle" to conduct the sinoatrial rhythm to the ventricles. He found that it made no difference whether the connecting strand was left at the site where the normal bundle would later develop (marked with an asterisk in fig. 2), or whether the strand was left on the opposite wall of the heart.

During the fifth and sixth weeks of human development, endocardial and epicardial connective tissue gradually encroaches on the myocardium at the A-V sulcus and invades this connecting zone. The separation of the atria and ventricles is ultimately completed all the way around the sulcus, as the primordial fibrous skeleton of the heart is formed. However, early in the sixth week of development (9 to 10 mm. human embryo), a compact cluster of cells makes its appearance in the posterior wall of the A-V canal, toward its right side.<sup>4</sup> This is the A-V node, in cellular continuity with the atrial muscle above and narrowing into the A-V bundle below. The bundle runs across the top of the thick interventricular septum, behind and under the dorsal endocardial cushion (fig. 3<sup>†</sup>). At this stage relatively little cytologic differentiation has yet occurred in either the node or

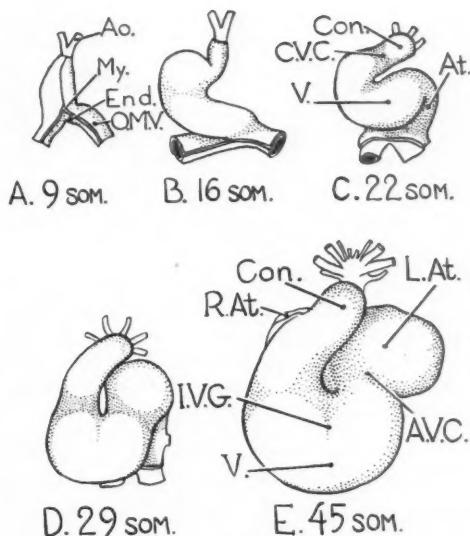


Figure 1

*Early stages in cardiac morphogenesis. In the chick the stages illustrated would be at about (A) 36 hours, (B), 46 hours, (C) 52 hours, (D) 62 hours, and (E) 3½ to 4 days. Equivalent stages would occur during the first 4 weeks of development in the human. At (L and R), left and right atrium; Ao., ventral aortic root; A.V.C., atrioventricular constriction; Con., conus arteriosus; C.V.C., conoventricular constriction; End., endocardial tube; I.V.G., interventricular groove; O.M.V., omphalomesenteric vein; My., cut edge of myocardium; V, ventricle. (Redrawn from Patten.<sup>69</sup>)*

bundle. The bundle, of course, is not invaded by the encroaching connective tissue and remains as the single fascicle of conducting fibers connecting the atria and ventricles.

Within a week or 2, the bundle branches arise, and the node and bundle become clearly distinguishable from the surrounding myocardium as pale-staining meshworks of cells with rounded nuclei, containing scattered and poorly striated myofibrillae (fig. 4). As they approach the apex of the heart, both the left and right bundle branches ramify into progressively smaller groups of fibers with much interlacing and "anastomosis."<sup>5, 6</sup> The cells of the branches, proximally, are similar histologically to those of the bundle itself, which in turn resemble nodal cells. As

\*Figure 2 reproduced from Patten: Univ. Michigan M. Bull. 22: 1, 1956.<sup>3</sup> By permission of the Michigan University Medical Bulletin.

†Figure 3 reproduced from Walls: J. Anat. 81: 93, 1947.<sup>4</sup> By permission of the Journal of Anatomy.

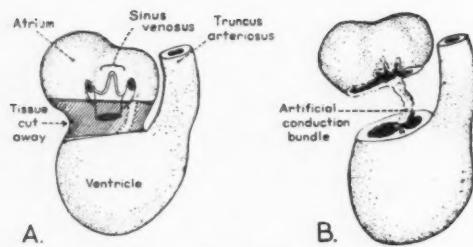


Figure 2

Heart of a 4-day chick embryo dissected to leave a connecting muscle strand between atrium and ventricle, simulating an ectopic A-V bundle. A. Dorsal view of heart with diagonal hatching indicating the tissue to be removed. B. Same heart after dissection (From Patten<sup>3</sup>).

the branches ramify distally, they become swollen and "watery," exhibiting a central juxtanuclear hyaline space, peripheral poorly formed myofibrils, and scattered mitochondria; that is, they become typical Purkinje fibers (fig. 5). In the human embryo, such fibers do not appear until rather late, probably between the tenth and fifteenth week of development (60 to 100 mm.)<sup>4, 7</sup> However, by the end of the fetal period, a complex, interlacing basketwork of specialized fibers invades all parts of the ventricular musculature. A reconstruction of the entire ventricular conducting system is shown in figure 6, which is a retouched photograph of a model made by Lydia DeWitt.<sup>8</sup> It shows the A-V node, bundle, branches, and the major ramifications. The fine "terminal" Purkinje fibers are not shown. (Throughout this paper, the tissue comprising the S-A and A-V nodes, the bundle, branches, and Purkinje fibers, will be referred to as "conductive tissue.")

The consequence of all this embryonic histodifferentiation is the presence of two readily distinguishable types of fibers in the adult heart of most mammals and birds. Some of their respective properties are summarized in table 1, which compares certain histochemical and cytologic properties of typical myocardial, conductive, and embryonic heart cells.

In spite of their manifest differences, we

should remember that many similarities also exist between myocardium and conductive tissue. Both types of fibers exhibit intercalated discs, striated myofibrils, sarcosomes, and other cytoplasmic inclusions characteristic of muscle. Both contain antigenic combining groups of cardiac myosin.<sup>21</sup> Moreover, it is now generally accepted that they are also physiologically similar, in that all heart tissue is characterized by the 3 functional properties: contractility, conductivity, and spontaneity. Let me quote from 2 representative sources on this matter. In a recent paper on the "Microanatomy of Muscle," Walls<sup>22</sup> states that:

"Cardiac muscle tissue is found only in the heart and surrounding the mouths of the great veins which enter it. In some ways it resembles both voluntary and smooth muscle, yet in its rhythmic, unceasing activity from early embryonic life until death, it stands alone." (p. 52).

And, in his chapter in Fulton's Physiology, Nahum<sup>2</sup> calls our attention to the fact:

"that all parts of the musculature of the heart are inherently capable of autogenic rhythmic discharge, and that the normal sequence of excitation is made possible only because the sinoatrial node possesses this capacity in a higher degree than any other region." (p. 672).

Thus we are told that the properties of cardiac muscle distinguish it clearly from other types of muscle, and also that heart muscle consists of a basically homogeneous population of fibers, all having essentially the same properties, showing only certain minor differences in degree in different regions.

The idea of the heart as a syncytium of fibers in cytoplasmic continuity tends to foster this concept of homogeneity.

#### Cellular versus Syncytial Structure

Early histologists considered heart muscle as cellular in structure and interpreted the intercalated discs as junctional zones of apposed cell membranes, staining heavily as a result of intercellular cement substance. For example, Eberth,<sup>23</sup> to whom the first detailed description of the intercalated disc is usually attributed in 1866, regarded the discs as intercellular structures, not only on the basis

Table 1  
*Properties of Adult Myocardium, Conduction Tissue and Embryonic Heart*

	Myocard.	Cond. Syst.	Emb. Hrt.	References
<b>Cytology</b>				
Myofibrils				
Well-formed, densely, regularly packed	+	—	—	9, 10, 11, 12, 13, 14
Heavy, parallel striations	+	—	—	9, 10, 11, 12, 13, 14, 15
Run parallel to cell axis	+	—	—	9, 10, 11, 12, 13, 14, 15
"Prodromal" striation pattern	—	+	+	9
Cytoplasmic inclusions				
Loose, non-striated filaments	—	+	+	9, 10, 11, 12, 14
Well-formed granular endoplasmic retic.	+	—	—	9, 10, 11, 15
Mitochondria few and juxtanuclear	—	+	?	9, 10, 11, 12, 14, 15
Rows or clusters of Palade granules	—	+	+	9, 11, 14
Numerous glycogen granules	—	+	+	9, 11, 15, 16, 17
Intercalated discs				
Interdigitating and complex	+	—	—	9, 13, 14
<b>Histology</b>				
Stain heavily with:				
Hematoxylin-eosin	+	—	—	15, 17
Silver impregnation	+	—	?	17, 18
Iron hematoxylin	+	—	—	17
Mallory trichrome	+	—	?	18
Glycogen Schiff reaction	—	+	+	16, 17, 19
Masson's trichrome, brick red or purple gray	BR	PG	?	17, 20

of their histologic appearance and their strong reaction with silver nitrate (known to stain cell membranes), but also because of the tendency of macerated heart muscle to fragment along the intercalated discs. Later, Ranvier<sup>24</sup> also described mammalian myocardium as being composed of individual rhomboidal branching cells with centrally placed nuclei.

It was not until after the turn of the century that Heidenhain<sup>25</sup> and Godlewski<sup>26</sup> independently enunciated clearly the hypothesis that the heart was syncytial in nature. These workers felt that the discs represented either contraction artifacts or the sites of sarcomere differentiation. Perhaps the strongest proponent of the syncytial nature of adult myocardium was H. E. Jordan, who, over a period of a decade or more, published observations and strenuous arguments in support of this idea.<sup>27, 28</sup> (It is interesting to note, however, that even Jordan was compelled to admit to the cellular structure of Purkinje tissue and of embryonic heart.)

The controversy over syncytium versus cells recurred periodically in the literature of the first half of this century, as some microscopists continued to make observations corroborating the earlier cell view.<sup>29</sup> However, most investigators during this period gradually tended to accept the syncytial hypothesis and even extended it to the conductive tissue.<sup>30</sup>

Especially important in this trend toward the idea of a cardiac syncytium were the early demonstrations of the "all-or-none" response of heart tissue by Bowditch,<sup>31</sup> Woodworth,<sup>32</sup> and others. It was felt that this finding could best be explained on the basis of protoplasmic continuity between all cardiac fibers. During the last decade, also, confirmatory electrophysiologic evidence has been obtained. In 1951, Curtis and Travis<sup>33</sup> reported that bundles of Purkinje fibers in the false tendon of the ox heart responded to electric stimulation in an all-or-none manner, that is, the demarcation potential, measured between the cut end and the surface, remained at con-

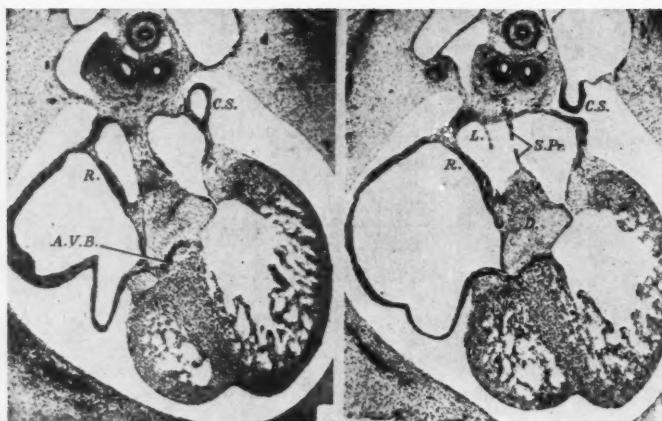


Figure 3

Section through the heart of a 10-mm. human embryo. Left. The bundle (A.V.B.) may be seen arising from the A-V node and passing into the base of the dorsal endocardial cushion. Right. Continuation of the bundle down the left side of the interventricular septum. R=right sinus valve; L=left sinus valve; C.S.=coronary sinus; S.Pr.=septum primum; D=dorsal endocardial cushion. (Hematoxylin-eosin stain.  $\times 30$ .) (From Walls.)

stant amplitude with varying strengths of suprathreshold stimuli. Thus the fibers appeared to behave syncytially. This was further supported by measurements of specific core resistance of Purkinje fibers by Weidmann.<sup>34</sup> He showed that, although the resistance of the cylindrical membrane surrounding a fiber was of the same order as that of muscle or nerve, the core resistance (i.e., the longitudinal internal resistance) was not appreciably higher than that of the sarcoplasm. He concluded that the intercalated discs do not form a significant barrier to ionic movement along the length of the fiber.

In spite of the evidence of homogeneity (and even identity) of all fibers, Thomas Lewis (1925), after some 20 years of pioneering work in cardiac electrophysiology, proposed his "law of cardiac muscle."<sup>35</sup> In this statement, he noted that there are different kinds of heart cells: those characteristic of myocardium, and typical Purkinje fibers. Because of the prevailing opinions, this idea was not strongly favored, and few references to it appear in recent decades.

In the last few years, however, since electron microscopy has been applied to this problem, it has been shown repeatedly and without exception that the idea of a cardiac syncytium lacks a sound anatomic basis. Perhaps a dozen electron microscopists, using ever more sophisticated techniques, have found

that the intercalated disc does consist of a pair of apposed cell membranes, covered with densely staining granules. The myofibrils do not cross the intercalated discs, nor is there any other evidence for any kind of protoplasmic continuity across the membranes. Each elongate cell of a myocardial fiber is consistently observed to be surrounded by an intact plasma membrane (for recent review of evidence see<sup>36</sup>). This is true in both myocardium and conductive tissue.<sup>9, 12</sup> Moreover, the pattern of development of the intercalated discs in cardiac myoblasts, at the sites where the myofibrils are interrupted by cell membranes, also supports the idea of a cellular structure.<sup>10, 13</sup> Thus, neither in the embryo nor in the adult heart; neither in myocardium of cold-blooded<sup>37</sup> or warm-blooded vertebrates; nor in nodal or conductive tissue is there a satisfactory anatomic basis for the notion of syncytial structure.

#### A Functional Syncytium?

The studies of Curtis and Travis<sup>33</sup> and of Weidmann<sup>34</sup> noted above, led to the use of the concept of heart tissue as a "functional syncytium." By this it is implied that even though cardiac fibers do not, in fact, exhibit protoplasmic continuity, under certain conditions they behave as if they did. For example, in small cultured fragments (50 to 100 microns in diameter) of embryonic heart tissue, the membrane effects of a polarizing

current applied through one of a pair of intracellular microelectrodes can be recorded in any cell of the fragment, even when the recording electrode is as much as 100 microns from that used for stimulation, and visible cell boundaries separate the two.<sup>38</sup> Moreover, synchronous and identical diastolic depolarization and action potentials can be recorded from all cells of such a spontaneously beating clump.

However, other physiologic evidence is accumulating, which suggests that individual cells, or at least small regions of cardiac tissue, are capable of separate activity, independent of neighboring cells or regions. Embryonic heart, for example, can be disaggregated with trypsin into small cell clusters, from which transmembrane resting and action potentials can be recorded. Such recordings are similar in all respects to those obtainable from cells in the intact heart.<sup>39</sup> Thus the electrical activity of these cells is clearly unaffected by cellular dissociation. Moreover, in the cooled adult mammalian heart<sup>40</sup> or frog heart perfused with hypertonic Ringer-sucrose solution,<sup>41</sup> it is possible to record spontaneous action potentials from one fiber, while a neighboring fiber is completely quiescent, or fires at a different rate. Similarly, Cervoni, West, and Falk,<sup>42</sup> recording intracellularly from rabbit atrial preparations, found that, when such a preparation was stimulated at 7 to 8 beats/sec., atrial cells responded to every stimulus, while nearby cells from the S-A node responded only to every other stimulus. At a time when a nodal cell was still in its refractory period, a neighboring atrial cell was fully repolarized and capable of firing.

Thus the weight of anatomic evidence appears to support the concept of the heart as a population of discrete cells, separated transversely by intercalated discs. Whatever the normal mechanism of transmission of the impulse across the discs, physiologic studies show that such transmission can readily be disturbed, with the result that the individual cells or contractile units can exhibit their independent nature. The histologic differ-

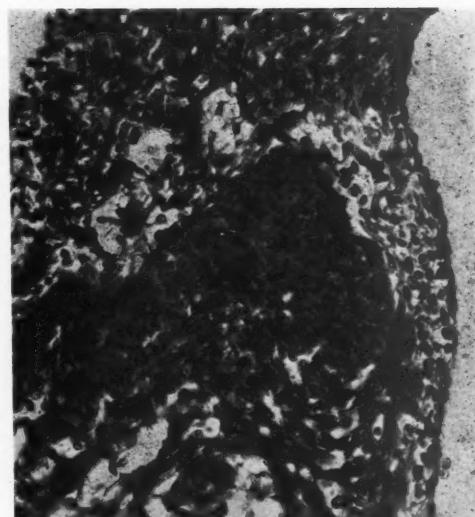


Figure 4

*Cross section of the A-V bundle (B) and root of the right bundle branch (RB) in the crest of the interventricular septum (IVS) of a 7-day chick embryo heart. (Phosphotungstic acid hematoxylin.  $\times 300$ .)*

entiation described above would further suggest that there are at least two distinct cell types: that making up the bulk of the myocardium, and a clearly different, perhaps more embryonic type, which constitutes the conductive system. (Space does not permit mention here of the evidence for intermediate types<sup>20, 43</sup>) It is evident that processes of cell differentiation leading to such histologic differences must be based upon changes at the biochemical level. Is there, then, any evidence of biochemical or physiologic differentiation between myocardial and conductive cells?

#### Biochemical Differentiation

Quantitative biochemical differences between myocardial and conductive cells are many (see Schiebler,<sup>17</sup> for review). For example, conductive tissue contains more choline acetylase than does myocardium.<sup>44, 45</sup> Healthy adult myocardium is quite low in glycogen,<sup>46</sup> whereas the rich concentration of glycogen in conductive tissue has been noted repeatedly<sup>17</sup> and can even be seen with in-

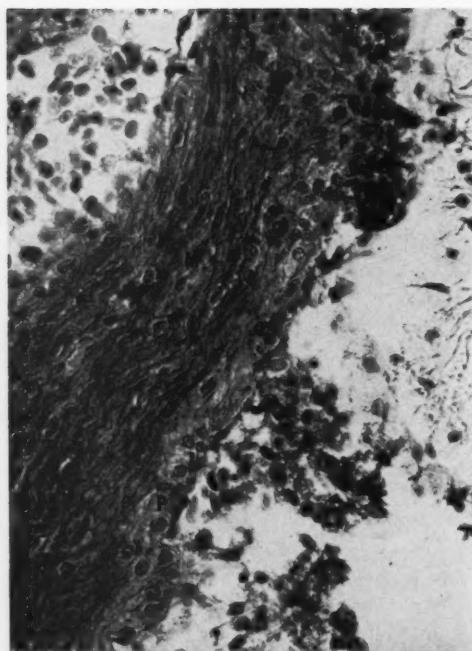


Figure 5

Subendocardial Purkinje cells (P), compared with myocardium (M), in a newly hatched chick embryo heart. (Phosphotungstic acid hematoxylin.  $\times 400$ .)

travital staining in the living heart.<sup>47</sup> Its abundant supply of glycogen would suggest that conductive tissue might be highly dependent on glycolytic metabolism, perhaps in lieu of a strong oxidative cycle. This idea is supported by the finding that conductive tissue has a much lower succinic dehydrogenase activity than does myocardium, and has a total oxygen consumption rate only one-fifth as great. It is also resistant to cyanide and to anoxia.<sup>19</sup>

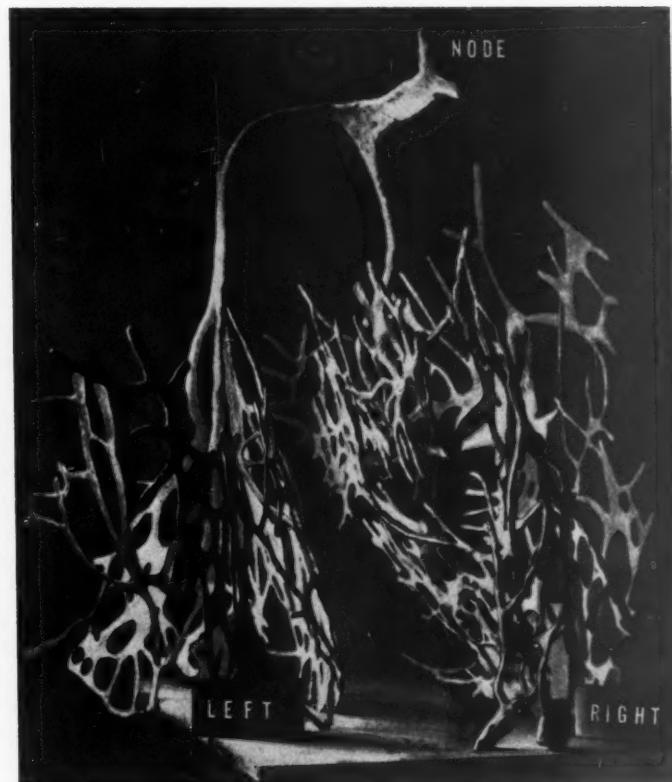
There are 2 enzymes or enzyme systems in conductive tissue that appear to be qualitatively absent from myocardium in most species. (It is recognized that such a claim, based upon negative evidence, is valid only within the limits of presently available techniques.) Although nonspecific cholinesterases are abundant in all heart cells, Mommaerts et al.,<sup>48</sup> were able to characterize an enzyme

from the A-V bundle and bundle branches of beef heart as a specific cholinesterase, similar to that characteristic of nervous tissue. More recently, Carbonell<sup>49</sup> confirmed this and localized the enzyme histochemically to cells of the conduction system in hearts of the human, dog, cat, guinea pig, rabbit, rat, sheep, and cow. Only in the rabbit were equivocal results obtained, in which scattered cells in the myocardium exhibited esterase activity that was nonspecific in its substrate specificity, but was inhibited by eserine (1 of the criteria for specific cholinesterases). In the other species mentioned, specific cholinesterase was limited to cells of the conduction system (fig. 7\*).

The second enzymatic activity seen exclusively in conductive tissue is that responsible for a phenomenon termed "aberrant lipogenesis," and suggests a distinctive fat metabolism in this tissue. Recently Kuwabara and Cogan<sup>50</sup> have shown that Purkinje tissue can synthesize sudanophilic fat when incubated in serum supplemented by oleic acid, a capacity not shared by ordinary cardiac muscle. This property depends upon the presence of (1) an intracellular sulphydryl-requiring enzyme system, (2) serum, and (3) oleic acid or sodium oleate as a substrate. It is clear from their work that cells of the conductive system exhibit this enzyme, whereas those of the myocardium apparently do not.

It is interesting to note that many of the biochemical and metabolic characteristics distinguishing conductive tissue from myocardium are the same as those differentiating embryonic from adult heart. Heart muscle of the early embryo is rich in glycogen,<sup>46, 51</sup> and highly resistant to anoxia.<sup>52</sup> It is also low in succinoxidase<sup>53</sup> and cytochrome oxidase<sup>54</sup> activity, and is relatively insensitive to cyanide poisoning.<sup>55</sup> Conductive tissue is thus distinctly "embryonic," in sharing some of the cytologic, histochemical and biochemical characteristics of primitive heart. At the

\*Figure 7 reproduced from Carbonell: J. Histochem. 4, 87, 1956.<sup>49</sup> By permission of the author and The Williams & Wilkins Co.



**Figure 6**

*The atrioventricular conducting system of the calf heart, retouched to clarify its 3-dimensional aspects. (Modified from DeWitt.<sup>8</sup>)*

same time, however, it shows definite signs of having differentiated along an independent path, developing a distinctive morphology and biochemistry, with at least two enzymes not shared with myocardium.

#### **Physiologic Differentiation**

Does the physiology of myocardium and conductive tissue show a similar pattern; that is, do these 2 tissues exhibit some functions that differ only in degree, and others that are totally restricted to 1 or the other?

One well-known quantitative physiologic difference between myocardium and the conductive system is in conduction velocity. Since the observations of Erlanger,<sup>56</sup> it has been accepted that the conduction tissue is capable of transmitting an impulse more rapidly than myocardial muscle (with the exception of certain specific areas, as at the atrionodal junction). In 1959, Draper and

Mya-tu<sup>57</sup> measured the rate of conduction through specialized fibers in false tendons, and in the bundle branches, at a velocity of 2 to 3 meters/second. In contrast, strips of parallel myocardial fibers not containing Purkinje tissue were able to conduct the stimulus at a rate of only 0.5 to 0.6 meter/second. Clearly this represents a differentiation of new membrane characteristics by the specialized fibers to permit such rapid conduction, since embryonic myocardium starts out with a low conduction rate comparable to that which is apparently retained by adult myocardial muscle.<sup>58</sup>

A second difference between myocardial and conductive cells, at a physiologic level, lies in the configuration of their action potentials. Intracellular recording from cells in various parts of the conductive system reveals action potentials that, when compared with those from myocardial fibers, have a

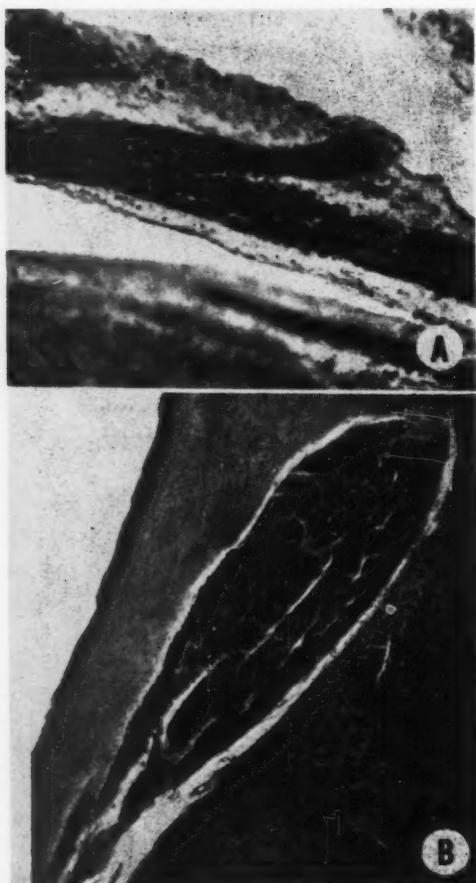


Figure 7

Specific cholinesterase activity in conduction tissue of the adult human heart. A. Subendocardial Purkinje fibers. B. A-V bundle and left branch. (From Carbonell.<sup>49</sup>)

low amplitude with little or no overshoot, a low rising velocity of the upstroke, and long duration with a rounded plateau. In contrast to the steady resting potential common to myocardial fibers, recordings from pacemaker regions also show a characteristic slow diastolic depolarization of 10 to 20 mv,<sup>48, 59</sup> termed the "prepotential" by Bozler.<sup>60</sup> The prepotential, in association with pacemaker activity, differentiates extremely early in cardiac development, long before specialized nodal or conduction tissue is histologically

distinguishable. Recently Meda and Ferroni<sup>61</sup> have been able to insert intracellular electrodes into hearts of chick embryos only a few hours after initiation of the beat. Even at these early stages (see fig. 1 A and B), cells showing a slow diastolic depolarization were found in the S-A region, while cells in the ventricle showed steady resting potentials similar to those from adult myocardium (fig. 8\*). Thus, embryonic ventricular cells even in the 13-somite chick embryo, do not originate their own beat but are stimulated by cells in more posterior regions, which are acting as pacemakers.

This leads us to the final physiologic property that I should like to compare between myocardium and conductive tissue: the property of spontaneity, or pacemaker function. We have seen that in the embryonic heart, ventricular cells do not initiate their own beat but are driven by other cells. It is commonly accepted, also, that in the adult heart the contraction stimulus arises in the definitive pacemaker, the S-A node, and is transmitted to all parts of the heart via the fibers of the conduction system. Yet, as noted earlier, the idea that all heart muscle is characterized by the property of spontaneity seems to pervade the thinking of most cardiac physiologists, and it is stated explicitly in textbooks and other reference sources. Evidence appears to be accumulating, however, which suggests that the ability to generate bioelectric potentials, i.e., the pacemaker function, may not be a property common in varying degrees to all heart cells, but may be limited exclusively to cells that differentiate into some component of the conduction system. Myocardium, in contrast, appears to be completely quiescent until stimulated by an extrinsic source, in a manner analogous with skeletal muscle.

It should be emphasized that a systematic analysis of this problem has not been carried out, and decisive experiments are few. However, I should like to consider some of the

\*Figure 8 reproduced from Meda and Ferroni: Experientia 15: 427, 1959.<sup>61</sup> By permission of Experientia.

relevant data. In 1910 Erlanger<sup>62</sup> showed that equal-sized strips of cat atrium, isolated *in vitro*, may be spontaneous or not, depending on what region of the atrial tissue is included. Strips of the left atrium frequently remained quiescent, while similar areas of right atrium, or of left atrium connected to the interatrial septum, usually showed spontaneous activity. We now know that those areas which Erlanger found it necessary to include in a strip if it was to beat spontaneously are the regions from which pacemaker prepotentials have been recorded; namely, the interatrial septum, the crista terminalis, the entrance of the superior vena cava, and the S-A ring bundle.<sup>43, 63</sup>

Evidence that spontaneity is limited to a portion of the cells of the heart arises also from observations made on such cells in tissue culture. Cavanaugh<sup>64</sup> disaggregated 4- to 6-day embryonic chick hearts. She found that immediately after the cells attached to the glass substratum only 9 per cent showed spontaneous activity. Within the first day or two after the cells had spread and established extensive filopodial interconnections, the proportion pulsating rose to about 50 per cent. Moreover, as interconnections were established in the culture, cells that had been quiescent, or beating independently, could be influenced by a dominant neighbor to contract in synchrony with it. Other examples of such pacemaker activity by embryonic cells or heart fragments exist in the literature,<sup>58, 65, 66</sup> and Harary and Farley<sup>67</sup> have recently shown similar behavior on the part of dissociated cells of young (nonembryonic) rat hearts. Thus it seems reasonable to conclude that in neither embryonic nor adult heart is the majority of cells capable of spontaneous contraction. It is recognized, however, that this conclusion may be premature when applied to the embryo, in view of the apparent lability of pacemaker function in immature myocardium. Cells in the embryonic ventricle *in situ* do not show pacemaker prepotentials.<sup>61</sup> However, cell clusters from this tissue, in culture, beat

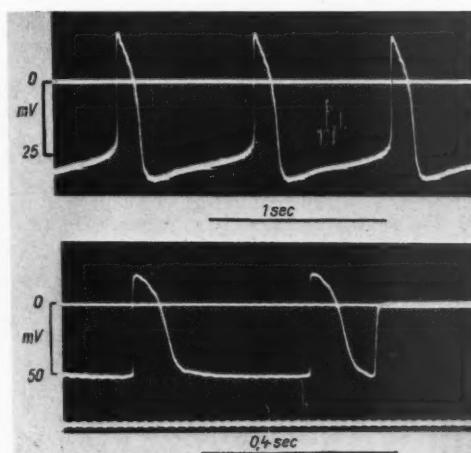


Figure 8

*Action potentials recorded from the heart of a 42-hour (13-somite) chick embryo. Top. Electrode inserted in the posterior end of the heart tube, in the presumptive atrial region; note the diastolic depolarization of approximately 10 mV. Bottom. Electrode inserted in the primitive ventricle; note the greater amplitude and shorter duration of these action potentials, as measured by the amplitude and time calibrations shown. (From Meda and Ferroni.<sup>61</sup>)*

spontaneously and do exhibit slow diastolic depolarization.<sup>38</sup> Thus, pacemaker activity in ventricular cells may be lost or suppressed during development of the intact heart but may be regained by at least some cells when isolated and subjected to culture conditions.

In adult heart, however, spontaneity does appear to reside exclusively in those cells that have differentiated into components of the conduction system. This argument has gained substantial strength from the recent observations of Draper and Mya-tu.<sup>57</sup> These investigators confirmed the findings of many others<sup>68</sup> by showing that muscle slips or papillary muscles from various mammalian hearts, maintained *in vitro*, are frequently not spontaneously active. However, they also noted that when such spontaneity is displayed, it "can always be traced to the presence of Purkinje tissue in the sample" (p. 107<sup>57</sup>). For example, in one group of 18 ventricular muscle slips, 9 did not beat spontaneously,

yet all responded vigorously to external stimulation for many hours and showed normal resting and action potentials. These muscle slips were serially sectioned for histologic examination. Those that had not beat spontaneously showed no evidence of Purkinje tissue, while specialized fibers were found in all those that were active. Thus, we may conclude that in adult heart, if conductive cells are present to initiate an impulse (and possibly a critical number is required), we see "spontaneous" activity. Without these cells the heart muscle, though healthy and completely responsive, remains quiescent.

#### Conclusions

When information obtained from studies of the structural, chemical, and functional properties of the A-V conducting system of the adult and embryonic heart is considered, different conclusions may be drawn than those arrived at by a purely physiologic approach. Though not yet firmly established by decisive experiments, these conclusions appear to be reasonable in view of the evidence presented. They may be stated as follows: (1) Cellular differentiation during embryonic development of the heart leads to the presence of 2 distinct populations of cells: typical myocardial cells, comprising the bulk of the musculature of the heart, and the specialized cells of the conductive tissue. (2) Although cells of the conductive system do exhibit certain of the properties of embryonic myocardium, the two tissues in the adult may be distinguished by characteristic and unique properties, at the histologic, biochemical, and physiologic levels of investigation. (3) The differentiation of pacemaker function occurs very early in cardiac development. Within hours after initiation of the beat, and perhaps earlier, cells of the ventricle do not originate their own beat but are stimulated by other cells functioning as pacemakers. (4) In the adult heart, myocardial cells appear to be completely quiescent until stimulated by an extrinsic source. Under normal circumstances, this stimulus source is a pacemaker cell (or cells) of the

S-A node, or some other part of the conduction system.

#### Acknowledgment

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# Cardiac Myosin and Congestive Heart Failure in the Dog

By ROBERT E. OLSON, PH.D., M.D., ERIC ELLENBOGEN, PH.D.,\*  
AND RAJA IYENGAR, PH.D.

Chronic congestive heart failure has been produced in dogs by surgical induction of valvular disease. Cardiac myosin was isolated from the normal dogs and from dogs with congestive heart failure and characterized. Physicochemical properties of the cardiac myosins were determined by measurements of velocity sedimentation, partial specific volume, rate of diffusion, limiting viscosity number, light-scattering behavior, and ATPase activity. The measurements show that normal cardiac myosin (myosin C) has a molecular weight of 225,000, whereas myosin from the failing heart (myosin F) has a molecular weight of 690,000. This change in molecular weight occurs without a marked alteration in amino acid composition and suggests that end-to-end trimerization of normal cardiac myosin occurs in association with congestive heart failure in the dog. There was no significant change in ATPase activity.

FOR THE PAST several years our laboratory has been engaged in the systematic study of cardiac metabolism in the dog in various conditions of malnutrition, endocrine imbalance,<sup>1</sup> and surgically induced valvular heart disease.<sup>2</sup> Of particular interest has been the study of the contractile proteins of the heart in various states, including experimental chronic congestive heart failure.

This study was undertaken because of the inability of many investigators,<sup>3</sup> including ourselves,<sup>4</sup> to discover in either dogs or man with heart failure any evidence of a biochemical defect in the uptake of substrates from the coronary blood, the oxidation of these substrates to carbon dioxide and water, or in the process of oxidative phosphorylation.<sup>5</sup> The shortening of the myofibril in the contractile process involves the interaction of at least 2 contractile proteins, myosin and actin, with ATP. Hence, in view of the above negative evidence, it seemed important to study the properties of these proteins in hearts of animals in various states of cardiac compensation. Since appropriate samples of cardiac muscle from human subjects are very difficult to obtain immediately after death,

it was necessary to study this problem in an experimental animal. This report presents the results of studies of the physicochemical properties of cardiac myosin isolated from normal dogs, from dogs with sodium retention after ligation of the inferior vena cava, and from dogs with chronic congestive heart failure from valvular disease. More detailed reports of these findings have appeared elsewhere.<sup>6, 7</sup>

## Methods

Normal mongrel dogs, immunized upon arrival from the kennel and known to be in good health through observations in our animal colony over several weeks, served as the normal control group for this study. Dogs with inferior vena cava ligation (ICL) having marked sodium retention and ascites served as the second control group. This ICL group was a particularly good control group because these animals possessed normal cardiac contractility in the presence of sodium retention and altered aldosterone metabolism.<sup>8, 9</sup>

In the first experimental group, congestive heart failure was produced in a series of animals by surgical avulsion of the tricuspid valve and stenosis of the pulmonary artery, essentially by the method of Barger, Roe, and Richardson.<sup>10</sup> This combination of surgical lesions, which was accomplished in 2 stages at 3-week intervals, produced congestive heart failure in 60 per cent of the surviving dogs, with an operative mortality of less than 15 per cent. These animals began to show clinical signs of congestive heart failure within 1 to 4 weeks after constriction of the pulmonary artery. These signs included oliguria, marked sodium retention, an increase in body weight with the appearance of ascites, and reduced exercise tolerance.

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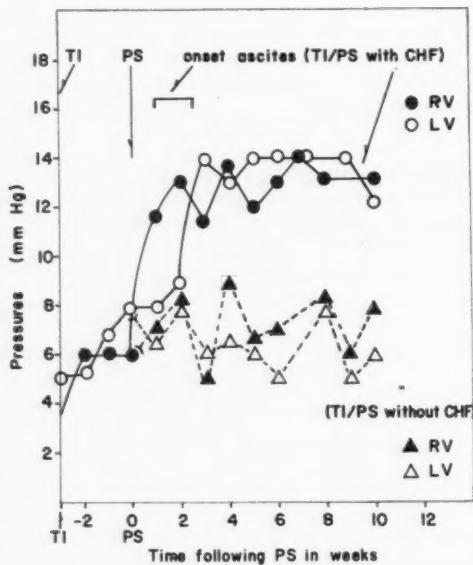


Figure 1

Ventricular end-diastolic pressures in dogs operated upon to produce tricuspid insufficiency and pulmonic stenosis. The time of each operation is noted. The end-diastolic pressures were measured in nonanesthetized dogs. The solid symbols are right ventricular pressures; the open circles are left ventricular pressures. The lower set of curves represents the pressures in animals that did not develop congestive heart failure.

In the second experimental group of dogs, primary left heart failure was produced by creating a mitral insufficiency followed by 1 of 2 aortic lesions. Aortic stenosis was produced by resecting the noncoronary aortic cusp and adjacent aorta with the formation of a bicuspid valve along the lines suggested by Carmella, Andersen, and Oropeza.<sup>11</sup> A constriction in the lumen of about 60 per cent was achieved. Aortic insufficiency was induced by punching holes in the non-coronary cusp with a circular valvulotome through the aorta according to Rosse and Morrow.<sup>12</sup> Postoperatively, the intraventricular pressures of many of these dogs were measured through the chest wall at intervals to determine the time of onset of failure. Statham gauges and a Sanborn visocardiograph were employed to record the pressures. Minimal morphine sedation was used to facilitate the procedure so that the dogs were quiet but awake and responsive. All of the animals sacrificed for characterization of cardiac myosin had been in failure for at least 6 weeks, as evidenced by elevated end-diastolic pressures.

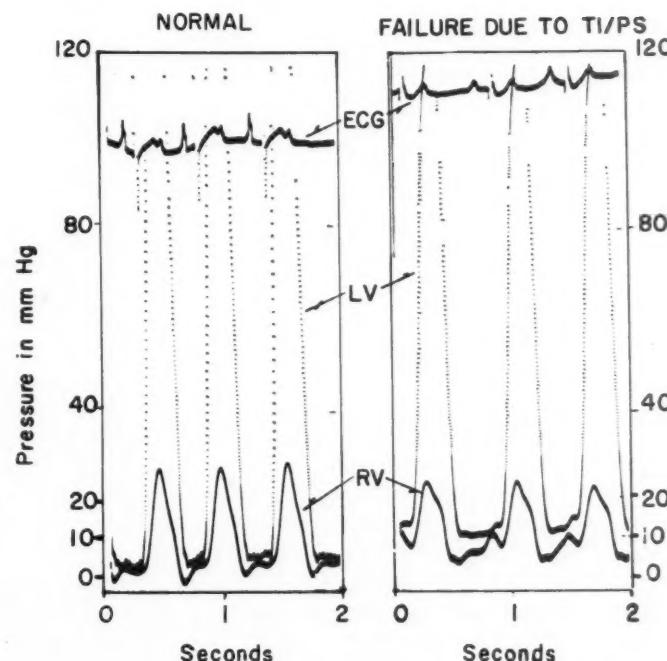
In order to describe the hemodynamic status of these animals prior to sacrifice, a light plane of anesthesia was induced in each animal by the intravenous administration of Nembutal: Dial-Urethane (1:1 v/v). Cardiac catheterization of the right heart and coronary sinus was accomplished, a femoral artery was cannulated, and pressure measurements were made in the femoral artery, right atrium, right ventricle, and pulmonary artery, by means of Statham gauge transducers with either a Sanborn twin visocardiograph or a Medical Electronics recorder. In some instances simultaneous tracings in right ventricle and left ventricle were recorded using a direct puncture of the left ventricle through the chest wall (see figure 2). Myocardial oxygen and carbon dioxide exchange and substrate extraction were measured by sampling simultaneously from the coronary sinus and the femoral artery. Coronary flow was measured by the nitrous oxide method.<sup>13</sup> Blood gases and substrates were measured by methods previously described and the results of studies of the metabolic activity of the failing heart are reported elsewhere.<sup>14</sup>

At the conclusion of the physiologic measurements the plane of anesthesia was deepened by administration of additional Nembutal to permit a thoracotomy and institution of artificial respiration. The pericardium was opened and the animal sacrificed by rapid excision of the beating heart. The organ was immediately chilled in deionized water in 1°C. Generally, a ventricular ectopic rhythm persisted until the heart had been immersed in the cold water for a few seconds. After being fully chilled to 1°C. the heart was dissected in a cold room to remove fat and connective and atrial tissue. Both right and left ventricular tissue were combined and minced in a meat grinder at 4°C., and the myosin was isolated by the method previously described.<sup>6</sup>

Cardiac myosin was isolated from dogs in the 2 control and in the 2 experimental groups and characterized by a study of velocity sedimentation, partial specific volume, rate of free diffusion, limiting viscosity number, light-scattering behavior, and ATPase activity. Partial specific volumes were determined by pyknometry. ATPase activity was determined at 25°C. on samples dialyzing against veronal buffer (pH 8.6) in order to remove phosphate ions. The method of Gergely<sup>15</sup> was employed and the ATPase activity expressed as  $Q_p$  (microliters of phosphorus liberated per milligram of myosin per hour). Sedimentation-velocity measurements were carried out in a Spino Model E analytical ultracentrifuge. Solutions above 0.2 per cent protein were analyzed in the conventional manner from schlieren patterns. More dilute solutions were

Figure 2

Tracings of right and left ventricular pressures are shown for a normal dog in the panel on the left and for a dog in congestive heart failure caused by TI/PS in the panel on the right. An electrocardiogram is included in each case for reference. The end-diastolic pressures in both the right and left ventricles are elevated in the animal with congestive heart failure.



analyzed by measurement of ultraviolet absorption, using a Spino analytrol to plot the density of the absorption bands. Sedimentation runs were carried out both at 24 and 4 C.; no dependence of sedimentation constant upon temperature or speed of rotor was noted. Diffusion constants were estimated both from boundary spreading observed in the ultracentrifuge and from free diffusion in the Spino Model H electrophoresis apparatus. Diffusion constants were calculated from schlieren patterns and from Rayleigh fringes respectively employing the method of second moments. The boundary-spreading coefficients were corrected for the concentration dependence of the sedimentation constant. Light-scattering measurements were carried out in a Brie-Phoenix light-scattering photometer equipped with a Brown recorder. The wave length chosen was a mercury blue line ( $436 \text{ m}\mu$ ). The refractive index increment was determined at the same wave length in a Phoenix differential refractometer and found to be 0.206 on a weight fraction basis. The solutions were clarified and measurements were carried out at 0, 45, 90, and 135 degrees at a temperature of 15 C. or less. The limiting viscosity number was obtained by measurement of the viscosity of solutions of different concentrations in an Oswald viscometer with a water time of about 180 seconds mounted kinematically in

an unsilvered Dewar flask at 2 C. Triplicate determinations were obtained with a maximal deviation of  $\pm 0.3$  second. Further details of these methods have been published.<sup>6</sup>

Three preparations of normal cardiac myosin and the myosin obtained from the failing heart were hydrolyzed in 6N HCl, and their amino acid was analyzed by the method of Moore and Stein.<sup>16, 17</sup>

### Results

#### Physiologic Studies

All the animals in this series with valvular disease showed generalized congestive heart failure at the time they were sacrificed for the study of cardiac myosin. Both the normal animals and the controls with inferior vena cava ligation showed no evidence of congestive heart failure.

In several of the animals operated upon, the development of failure was followed by measurements of end-diastolic pressures in the right and left ventricles. Results of a typical series of animals is presented in figure 1. After production of tricuspid insufficiency, end-diastolic filling pressure in the right heart was elevated from 2 to approxi-

Table 1  
*Hemodynamic Findings in Control and Experimental Dogs*

Condition	No.	Body* weight Kg.	Ascites L.	Cardiac index L./min./M. <sup>2</sup>	Coronary flow MI/100 Gm./min.	Oxygen usage MI/100 Gm./min.
Normal	12	19.9	—	2.59	87	10.8
		±1.1	—	±0.18	±6	±1.0
ICL	5	16.9	7.0	1.68	115	12.3
		±1.8	±2.0	±0.12	±12	±1.1
TI/PS (CHF)	11	18.8	6.0	1.89	75	10.0
		±1.1	±1.4	±0.24	±9	±1.6
MI/AI or AS (CHF)	2	21.3	—	3.36	119	14.5

\* Ascites-free

mately 6 mm. of Hg. After pulmonary stenosis was established, certain of the animals demonstrated a further rise in right ventricular end-diastolic, followed in 2 weeks by an elevation in left ventricular end-diastolic, filling pressure. The changes in the left heart followed the appearance of ascites. In the animals unsuccessfully operated upon (lower curve in figure 1) the end-diastolic pressures on the right side remain only moderately elevated and the pressures on the left side remain normal. Some of these later animals had transient ascites. Simultaneous left and right ventricular pulse tracings for a control dog and for 1 whose congestive heart failure was due to TI/PS are shown in figure 2.

The net body weight, amount of ascites, cardiac index, coronary flow, and oxygen usage by the myocardium for the 2 control and 2 experimental groups are shown in table 1. Both the animals with inferior vena cava ligation and those with tricuspid insufficiency showed a below-normal reduction in cardiac output, whereas those with left heart disease showed an increase in cardiac output. Coronary flow was not significantly changed from normal in any of the groups, and the oxygen usage by the myocardium was likewise within normal limits.

The evidence for congestive heart failure in these animals may be seen by examining table 2 in which the atrial and ventricular pressures are reported. Both the normal control animals and those with inferior vena

cava ligation controls showed essentially normal pressures in both chambers of the heart and no evidence of regurgitation into the right atrium. The animals with tricuspid insufficiency and pulmonary stenosis had a moderate right ventricular systolic hypertension (44 mm. vs. 30 mm. for the normal) and markedly elevated end-diastolic filling pressures. Regurgitation into the right atrium was marked in these animals, with the atrial systolic pressure reaching an average of 30 mm. of Hg. The left ventricle of these animals was also failing, indicated by the elevation of end-diastolic pressures in that chamber. In the 2 animals with left heart disease, there was evidence for left ventricular failure in terms of a marked elevated end-diastolic filling pressure. Some elevation of filling pressure was also noted in the right ventricle.

#### Physicochemical Studies

Results of the physicochemical studies are shown in figures 3, 4, 5, and 6, and are summarized in table 3. The data in figure 3 show the dependence of sedimentation constant (upon concentration) for myosin isolated from the control dogs (normal and ICL) and from those with congestive heart failure. The  $s_{20,w}$  for the control animals was  $6.16 \pm 0.13$  and for the animals in failure was  $6.50 \pm 0.01$ , a difference that is on the borderline of significance ( $p = < 0.05 > 0.01$ ). The concentration dependence of  $s_{20,w}$  is linear. The slopes of the sedimentation curves from normal and failing myosin are significantly

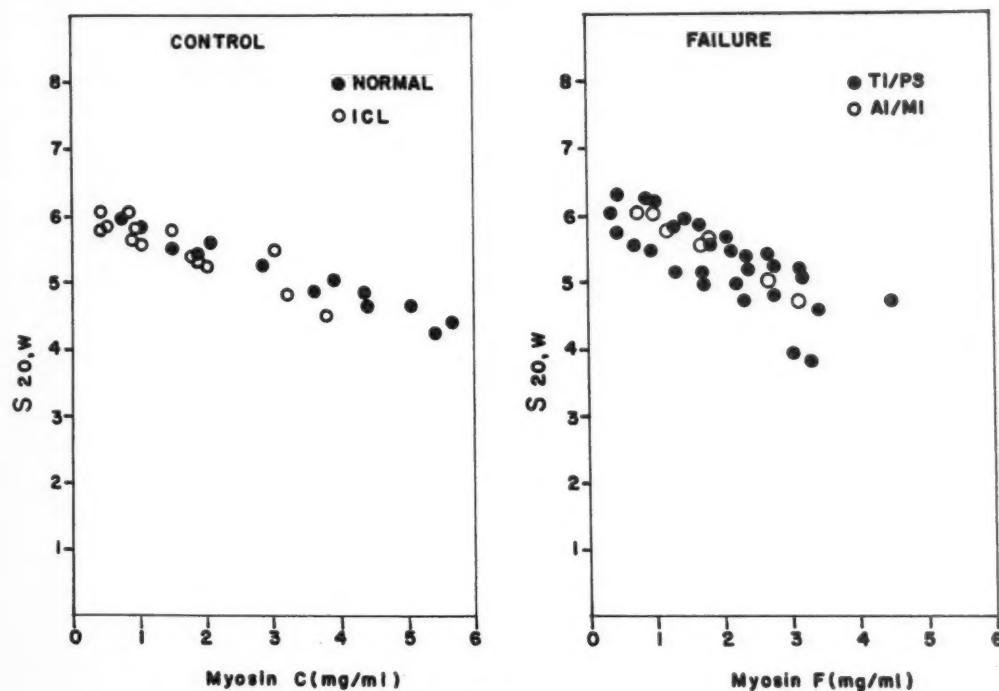


Figure 3

Sedimentation constants of dog heart myosin as a function of concentration. The panel on the left shows the values for control animals. The open circles are animals with ascites resulting from inferior vena cava ligation. The panel on the right shows the values for animals with congestive heart failure. The solid circles are from animals with primary right heart failure and the open circles are from animals with primary left heart failure. Conditions: 56,100 rpm; temperature 4 C.; 0.6M KCl; pH 6.8.

different. The calculated slope for the normal control group was  $-3.10 \pm 0.16$ , whereas for the dogs with heart failure it was  $-6.66 \pm 0.84$  ( $p = < 0.01$ ). The diffusion measurements for normal cardiac myosin and myosin isolated from dogs with heart failure are shown in figure 4. The concentration dependence of  $D_{20,w}$  was more marked with normal cardiac myosin than with myosin from the failing heart. On extrapolation to zero protein concentration the value for  $D_{20,w}$  for the normal dogs was found to be  $2.46 \times 10^{-7}$  cm.<sup>2</sup>/sec., whereas that for the dogs with heart failure was  $0.82 \times 10^{-7}$  cm.<sup>2</sup>/sec. The molecular weight calculated for normal cardiac myosin from sedimentation constant, diffusion constant, and partial specific volume was 226,000;

for myosin from the failing heart it was 680,000.

In figure 5 are plotted the turbidities uncorrected for dissymmetry at 90 degrees against protein concentration obtained in the light-scattering experiments for myosin from normal and failing hearts. The zero intercept of  $H \times e$  is proportional to the reciprocal of the molecular weight.

After applying correction factors for dissymmetry to the respective intercepts, the molecular weight for normal cardiac myosin was found to be 270,000, whereas that for failing cardiac myosin was 760,000.

The changes in intrinsic viscosity are presented in figure 6. The shape of the plots

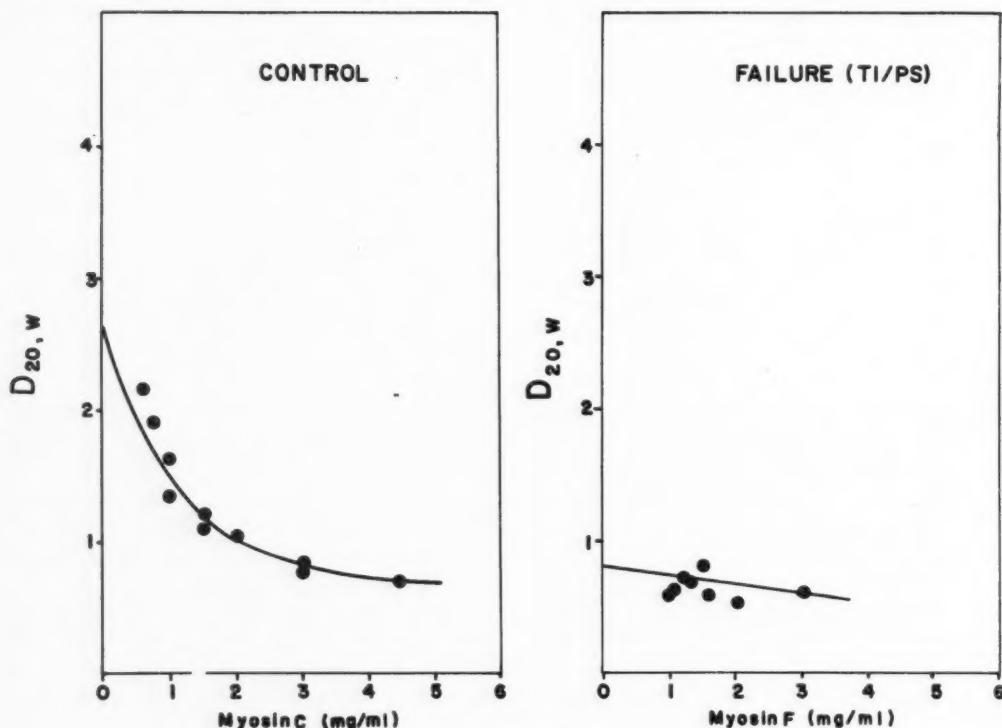


Figure 4

Boundary-spreading and free diffusion constants of dog-heart myosin as a fraction of concentration. The values in the left panel were obtained from normal animals; the values in the right panel from animals in congestive heart failure. Conditions: temperature 0.9 C.; 0.6M KCl; pH 6.8.

( $1/e \ln \eta/\eta_0$  vs. concentration) are markedly different for the 2 proteins. Normal cardiac myosin appears to disaggregate in very dilute solution and extrapolates to a limiting value of 50 c.g.s. units. On the other hand, myosin isolated from the failing heart shows a reciprocal behavior and appears to undergo aggregation in dilute solution to a high limiting viscosity number of 363 c.g.s. units. This behavior has also been noted by Davis et al.<sup>18</sup>

The ATPase activity of the myosins from normal and failing heart muscle were not significantly different. The value for normal myosin was  $382 \pm 68$  microliters P/mg./hr. vs.  $424 \pm 112$  for the myosin from the failing heart.

From analyses carried out thus far, it would appear that the amino acid composition

of myosin from normal heart, failing heart, and rabbit skeletal muscle are essentially indistinguishable on the basis of moles/100,000 Gm. protein. Representative data are shown in table 4. These data strongly suggest that the changes in molecular weight and other properties noted among the myosins represent changes in secondary and tertiary structure.

#### Discussion

The studies demonstrated conclusively that generalized cardiac failure can be induced in the dog by surgical production of tricuspid insufficiency and pulmonic stenosis. Elevation of end-diastolic filling pressures in the left heart as well as the right heart were noted in most of the dogs that have developed heart failure in our laboratory and all of these ani-

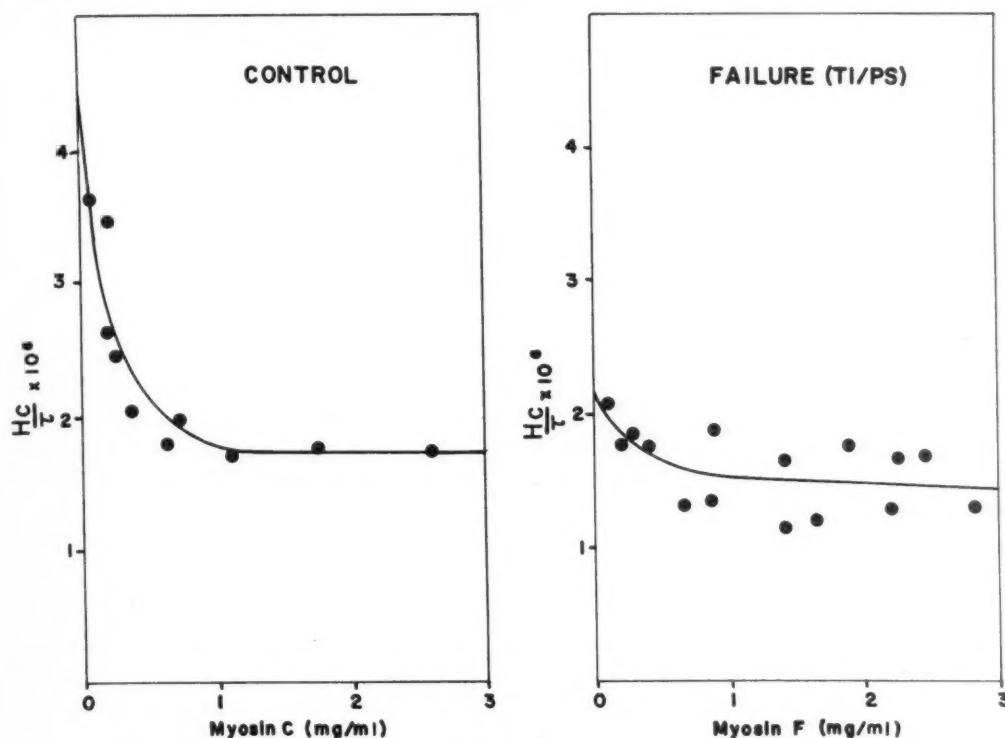


Figure 5

Light-scattering measurements on dog heart myosin as a function of concentration. The values in the left panel were obtained on preparations from control animals. The values in the right panel were obtained on preparations from animals with congestive heart failure. Conditions: temperature 12 to 15°C.; 0.6M KCl; pH 7.2.

mals selected for the study of cardiac myosin from the failing heart. This finding supports the studies of Barger, Roe, and Richardson<sup>19</sup> of heart-lung preparations made from hearts obtained from animals whose clinical cardiac failure was due to TI/PS. They observed that such hearts failed quickly in vitro in a Starling heart-lung circuit. Not only were the hearts unable to maintain normal output when the right atrial venous supply was increased, but also they were unable to maintain a normal output when the "Starling resistance" was increased. These authors concluded that "On the basis of two experiments, it would appear that as in cardiac failure in the human, the involvement of one chamber may predominate early in the dis-

ease, but with the progression of the disease decompensation of both chambers becomes apparent."

Our studies are in contrast to those of Davis et al.<sup>20</sup> in which the surgical production of tricuspid insufficiency and pulmonic stenosis lead to an elevation of right ventricular, but not left ventricular, end-diastolic pressures. These workers concluded that they had isolated right ventricular failure in their preparations. We can only conclude that the preparation they obtained was somewhat different from ours. They report that right ventricular systolic pressures were rarely increased in their animals, whereas these pressures were uniformly increased in ours. It is conceivable that the valvular dis-

Table 2  
*Intracardiac Pressures in Control and Experimental Dogs*

Condition	No.	Right atrial pressure		Ventricular pressures (mm. Hg)			
				Right		Left	
		Syst.	Mean	Syst.	Diast.	Syst.	Diast.
Normal	12	6.5	3.2	29.4	2.1	165	5.6
		±0.6	±0.7	±2.8	±0.8	± 4	±1.5
ICL	5	5.0	3.1	32.0	3.4	183	6.3
		±0.4	±1.1	±3.7	±0.4	±14	±0.3
TI/PS (CHF)	11	30.1	14.4	44.2	13.6	159	11.8
		±3.4	±0.1	±7.1	±1.2	± 7	±1.1
MI/AI or AS (CHF)	2	6.5	5.0	38.0	7.0	164	14.5

ease was more severe in our animals with extension of the defect in contractility to the opposite side, a phenomenon frequently seen in man<sup>21</sup> and expected on the basis of the anatomy of the heart.<sup>22</sup>

The molecular weight of normal canine cardiac myosin appears to be about 226,000 from data based on 3 independent methods carried out in our laboratory.<sup>6</sup> The value obtained from light-scattering is slightly highly (270,000), but this is typical of a technic that estimates weight-average rather than number-average molecular weight. On the other hand, the molecular weight obtained from studies of myosin from the failing heart is 690,000 from D, s, and  $\bar{v}$  and 760,000 from light-scattering measurements. The ratio of corresponding molecular weights for normal and failing myosin is 1:3. Since the amino acid composition is essentially identical it appears that the myosin from the failing heart is a trimer of that from the normal heart. If we designate normal cardiac myosin as myosin C (for cardiac) and myosin from the failing heart as myosin F (for failure), the transformation may be summarized as follows:

3 myosin C  $\rightarrow$  myosin F

The stimulus for this transformation seems to be chronic stretch. It may be that distortion of the myosin rodlet (thick filaments) in the chronically stretched myofibril may be instrumental in stimulating mild denaturation of normal myosin, which, in turn, results in polymerization. Since the ATPase activity of myosin F is not altered, the associa-

tion apparently does not involve the active site of this enzyme. Evidence for alteration in the myosin filaments in the failing heart are now being sought in our laboratory by electron microscopy.

It seems clear that the change observed represents an acquired molecular disorder that may account for the decrease in contractility of the failing heart. The phenomenon of polymerization of myosin has been observed to occur in vitro<sup>23</sup> during denaturation. The myosin of rabbit skeletal muscle may, furthermore, represent a polymer of a monomer of approximately the size of normal cardiac myosin. Kielley and Harrington<sup>24</sup> found that guanidine salts will depolymerize rabbit skeletal myosin to a monomer of molecular weight 219,000.

Benson<sup>25</sup> studied the effect of heart failure in dogs with tricuspid insufficiency and pulmonary stenosis upon the properties of actomyosin. The animals with experimental heart failure possessed a cardiac myosin with reduced ATP sensitivity, that is, a reduced change in the specific viscosity of actomyosin after addition of ATP. These investigators also found that the cardiac myosin peak from experimental dogs seemed to be more prominent in velocity sedimentation studies of actomyosin than in normals. They suggested that the combination of actin with myosin was less stable in the experimental dogs as compared with the controls.

Davis and his group<sup>26</sup> attempted to verify these findings but were unable to; they observed no difference in the ATP sensitivity

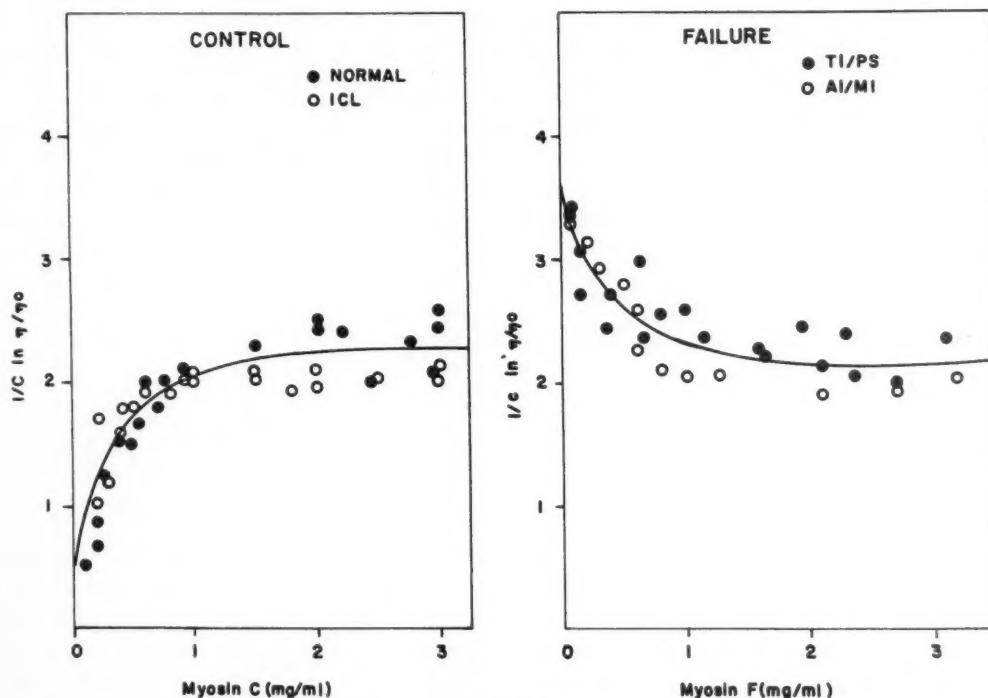


Figure 6

Viscosity measurements on dog heart myosin as a function of concentration. The values in the left panel were obtained on preparations of control animals. The values in the right panel were obtained on preparations from animals with congestive heart failure. Conditions: temperature 1 C.; 0.6M KCl; pH 6.8.

of actomyosin from normal heart muscle and heart muscle obtained from dogs with right heart failure resulting from tricuspid insufficiency and pulmonic stenosis. They did observe, however, that certain preparations of actomyosin from the failing right ventricle heart muscle did show a slow component (probably myosin) present in the sedimentation pattern that was not present in controls. In other words, the behavior of actomyosin from the failing heart was not identical with that from normals. Nevertheless, these investigators concluded that "these data do not support the concept that the contractile proteins are altered in experimental heart failure."

With regard to the work on actomyosin, it is probable that actomyosin formed during extraction of cardiac muscle by saline phos-

phate solution is a physiologic artifact. The electron microscopic studies of intact muscle fibers<sup>27</sup> suggest that actin and myosin are segregated in a particulate structure involving the thick and thin filaments in the living cell.<sup>28</sup> They appear to make contact only in a highly oriented manner during the contractile cycle. For this reason the studies of actomyosin, although controversial as noted, probably are not too informative about the state of the contractile proteins in the intact heart.

In the study reported more recently,<sup>18</sup> Davis and his co-workers undertook the characterization of cardiac myosin from normal dogs and from dogs with chronic congestive heart failure caused by tricuspid insufficiency and pulmonic stenosis. Their basic data are in fairly good agreement with ours as reported

Table 3  
Properties of Myosin from Normal and Failing Dog Heart

Constants	Cardiac Myosin	
	Normal	Failing
$s_{w20}^0, w$	6.16	6.53
$ds/dc$ (weight %)	-3.10	-6.66
$D_{w20}^0, w$	2.46	0.82
$\bar{V}$	0.73	0.72
$[\eta]$ (e.g.s. units)	50.0	363.0
$f/f_0$	2.15	4.56
M (from s, D <sub>w</sub> )	226,000	690,000
M (from light scattering)	270,000	763,000
a/b	24	80
Length (Å)	690	2,224
Width (Å)	28	28
ATPase (μL.P./mg./hr.)	382	424

above. They employed a narrower range of physicochemical methods to characterize the myosins, from normal and failing heart muscle than have been employed in the present study. For example, they did not carry out equilibrium sedimentation or light-scattering measurements on their preparations. Davis and co-workers did measure sedimentation coefficients over a reasonable range of concentration and their  $s_{w20}$  intercepts are in reasonable agreement with ours. We find a higher mean slope with the preparations from the failing heart, although the scatter is greater than with the normals. It is to be noted, however, that the sedimentation behavior is a relatively insensitive parameter for distinguishing normal and failing myosin because the sedimentation behavior is relatively unaffected by end-to-end aggregation.

Davis et al. admit, furthermore, the presence of impurities in some of their preparations stating that "in a few instances a very small boundary which sedimented faster than the principal myosin component was observed." If this is true, other impurities not distinguishable from the main peak, which could have modified sedimentation behavior at higher concentrations of the preparations from the failing heart, could have been present.<sup>29</sup>

The measurements of the diffusion con-

Table 4  
Distribution of Selected Amino Acids in Various Myosins (Gm. moles/10<sup>5</sup> Gm.)

Amino acid	Dog heart muscle		Rabbit skeletal muscle	
	Normal	Failure	Kominz, 1954 <sup>33</sup>	Bailey, 1948 <sup>32</sup>
Aspartic	83	79	85	67
Serine	39	39	41	43
Glutamic	149	144	155	150
Alanine	71	71	78	73
Valine	36	35	42	22
Phenylalanine	27	26	27	26
Lysine	84	78	85	81
Arginine	48	44	41	42

stant by Davis et al. were not carried out over a sufficient range of concentration to detect the concentration dependence in very dilute solution.<sup>6</sup>

The changes in intrinsic viscosity observed and described by us were also observed by Davis and co-workers. They were particularly notable in observations made by them on myosin from right ventricular tissue from dogs in congestive heart failure. An elevated intrinsic viscosity was consistently found. In a few preparations a high intercept was noted for the normals, although this was not as frequent nor as marked as in the animals with failure. Davis and co-workers choose to ignore these viscosity findings, which are in good agreement with ours, and concluded that their viscosity measurements were unreliable. "The possibility must be considered," Davis states, "that viscosity measurements at very low concentrations do not represent the true viscosity of the solution." The fact that their viscosity measurements were carried out at room temperature rather than at 1 C. makes it likely that denaturation of their normal preparation would occur with a higher frequency, which could account for the occasional higher intercepts noted in control preparations.

Without the additional support of measurements of light-scattering and equilibrium sedimentation behavior of these myosins, Davis et al. drew the conclusion that the molecular weight of cardiac myosin was in the range of  $5 \times 10^5$  and that there were no

differences between the normal and failing heart. We do not believe these conclusions are tenable in view of Davis's own data and particularly in view of the extensive work reported in this communication.

Whether or not there are differences in the animal preparations must be considered. Davis's animals were sacrificed without the benefit of an open chest and artificial respiration. Whether or not the degree of failure is different in the 2 groups of animals as evidenced by the apparently normal end-diastolic filling pressures on the right side in the Davis series can be resolved only by further experimentation. It is hoped that some reconciliation of these differences will be ultimately effected.

With regard to the question of generalized cardiac failure in dogs with TI/PS valvular disease, Benson<sup>30</sup> found that in vitro contractility of glycerol-extracted muscle strips from both the right and left ventricles of dogs with heart failure resulting from TI and PS was markedly reduced, as a depressed ventricular function curve similar to that observed *in vivo* could be constructed from the data. Since glycerol-extracted muscle retains little else than the basic contractile system and responsiveness to ATP, it seems reasonable to assume that the defective contractility observed by Benson and co-workers must be due to altered contractile proteins. Kako and Bing<sup>31</sup> observed a similar decrease in contractility of actomyosin bands prepared from failing human heart muscle post mortem when compared with control preparations.

It appears from these studies and ours reported herein that cardiac myosin is altered in physicochemical properties in association with congestive heart failure in the dog. The extent to which this is etiologic is not at this moment determined. Also, the extent to which this system is a model for the human with valvular disease is an interesting subject for speculation.

#### Acknowledgment

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# Contractile Proteins of Heart Muscle in Man

By RICHARD J. BING, M.D., AND K. KAKO, M.D.

This report deals with the contractile proteins of human muscle in congestive failure, and with the role played by the contractile proteins and by biochemical processes in the regulation of the mechanical function of the heart. The contractility of actomyosin bands prepared from heart muscle of patients who had died in congestive failure was diminished as compared to those prepared from normal hearts. This may have been the result of defective protein synthesis. The increase in heart rate was correlated with the activity of phosphorylase  $\alpha$  in heart muscle and with changes in carbohydrate intermediates (lactate, glucose-6-phosphate [G-6-P] and glycogen). The heart rates over 300 per minute were associated with a transient increase, followed by a decrease, in phosphorylase  $\alpha$  activity; glycogen diminished, while lactate and G-6-P increased. The oxidation-reduction potential in heart muscle became more negative. In the absence of myocardial anoxia, the increased rate of stimulation of the heart produced no alterations in either the concentration of carbohydrate intermediates or the phosphorylase  $\alpha$  activity. Alterations in function of the heart that come into play upon rapid changes of cardiac activity are the result of the integration of several diverse biochemical cellular reactions. The contractile proteins are but following the lead of the cellular elements concerned with the production of energy.

**C**ONTRACTILE PROTEINS of Heart Muscle in Man" is the topic assigned. Strict adherence to this title would limit the field to human heart muscle alone and would make this primarily a technical discussion. Therefore, little opportunity would be afforded for dealing with problems of broader physiologic significance. Consequently, although the title of this presentation will be adhered to in principle, it will also be used as a starting point to contrast the role played by the contractile elements and by biochemical processes in the regulation of the mechanical function of the heart.

## Contractile Proteins and Regulation of Cardiac Function

When one considers the properties of actomyosin in solution, it appears that this protein is most susceptible to the influence of adenosine triphosphate (ATP) and ions. In the presence of ATP, at low salt concentra-

tions, there is complete dissolution and dissociation.<sup>1</sup> Then, with an increase in potassium chloride, superprecipitation occurs.<sup>1</sup> As one increases the concentration of potassium chloride further, complete dissolution and dissociation suddenly take place again. Superprecipitated actomyosin forms a gel that can be compressed into threads.<sup>2</sup> Weber called these preparations thread models.<sup>2</sup> He showed that muscle models shorten by 80 per cent of their initial length and that they develop tension. Thus, the contraction of the living system and the contraction of these models agree in many points. We do, however, miss evidence of physiologic insight on the part of these models. They contract, they lift weights, and they develop tension, but their response to weight and their speed of contraction are uniform and not adjusted to the demands of the moment.

More than 10 years ago, with these considerations in mind, we undertook a study of the properties of models prepared from heart muscle both of animals and of man. The question that we tried to answer first was: Do models prepared from heart muscle retain some of the physiologic insight of the intact heart muscle; for example, what is the relationship between the tension devel-

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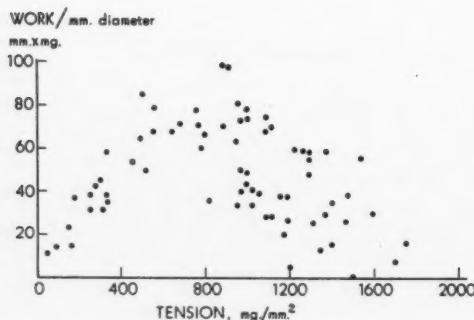


Figure 1

This shows the isotonic contractions of extracted heart muscle after the addition 0.8 per cent ATP. The work of the isotonically contracting muscle strip calculated per unit fiber (millimeter length per millimeter diameter) is related to the tension exerted on this preparation. The work performance increases with rising tension up to an optimal value and decreases when the tension becomes excessive. (From Taeschler and Bing.<sup>3</sup>)

oped and the work, and between the speed of contraction and tension? Dr. Taeschler, using the "fiber model" preparation of Szent-Györgyi (the glycerinated heart muscle fiber) found that the work of the extracted heart muscle increases with rising tension up to a maximal value and then decreases as this load is exceeded (fig. 1\*).<sup>3</sup> In this respect, extracted heart muscle reacts similar to fresh heart or skeletal muscle or, for that matter, to the whole heart.

This observation, which was interpreted as evidence that the molecular orientation of the contractile proteins of heart muscle during stretch determines the work performance, prompted us to extend our studies to the contractile elements of the human heart.<sup>4</sup> If our conclusions were correct, then similar techniques should enable us to accumulate evidence of an altered state of contractile proteins in heart muscle of patients who had died in congestive failure. From our metabolic studies, we had already reached the tentative conclusion that in heart failure, excluding

\*Figure 1 reproduced from Taeschler and Bing: Circulation Research 1: 129, 1953.<sup>3</sup> By permission of the American Heart Association, Inc.

such types as beriberi or thyrotoxic heart disease, the disturbance may be in the contractile proteins.<sup>5</sup> If one could prepare actomyosin models from failing human hearts, one might supplement the studies of Olson,<sup>6</sup> Davis,<sup>7</sup> and Benson,<sup>8</sup> who had attacked this problem by means of biophysical and physical-chemical techniques.

The success of this work depended on 2 unknowns: (1) are available actomyosin models suitable for this work, and (2), since the studies were to be based on the contractility of actomyosin bands obtained from hearts after the death of the patient, it would have to be shown first that characteristic properties of actomyosin do not change for a brief period after death. Dettli explored both these problems; in answer to the first question, he found that actomyosin threads, produced by the compression of surface spread fibers of the proteins, possess certain definitive disadvantages.<sup>9</sup> In a thick thread, such as the one prepared by Hayashi, most of the molecules within the thread are not exposed to immediate contact with ATP but must be reached by unequal diffusion of ATP from the bath.<sup>10</sup> In addition, the thickness of the thread varies a great deal, making it difficult to obtain uniform results. Dettli overcame these difficulties by compressing actomyosin, spread on the surface of a solution, into bands not into threads.<sup>9</sup> This afforded a more constant diffusion of ATP into the preparations and made them a useful tool in comparative experimental studies (fig. 2). Dettli also devised an apparatus for the measurement and recording of after-loaded isotonic contractions; in this system, refined by Kako, it is not necessary to handle the sticky actomyosin band directly; instead, the band can be loaded by moving the weighing spring of a torsion balance by the desired number of milligrams (fig. 3\*).<sup>4, 9</sup> Since the band contracts on the addition of ATP, the arm of the torsion balance follows the con-

\*Figures 3 and 4 reproduced from Kako and Bing: J. Clin. Invest. 37: 463, 1958.<sup>4</sup> By permission of the American Society for Clinical Investigation.

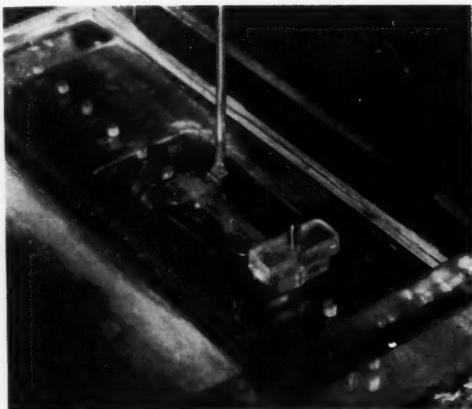


Figure 2

This illustrates an actomyosin band in the contraction chamber. One end of the band is anchored firmly, while the other is attached to the lever of a torsion balance.

traction. This initiates an electronic servo feedback mechanism, which moves the trough in a direction opposite to that of the contraction. Thus, the arm of the torsion balance is always kept in the equilibrium position and a counter force is produced that equalizes the tension of the thread.<sup>4</sup> The movements of the trough are recorded, and, without appreciable friction, it is possible to register shortening of the band at a magnification of 23 diameters.

The second problem, possible changes in the contractility of actomyosin after death, was studied by Dettli for the dog's heart and by Kako in actomyosin bands prepared from human hearts.<sup>4, 9</sup> Kako found that the contractility of actomyosin bands remained undiminished for at least 6 hours after the death of the patient.<sup>4</sup>

The way now appeared open for a comparison of the contractility of actomyosin bands prepared from the left ventricles of normal and of failing human hearts. The actomyosin bands prepared from heart muscle of patients who died in congestive failure were found to possess diminished contractility (fig. 4). It is not within the scope of this paper to dwell on the reasons for this dimin-



Figure 3

The Langmuir trough in which the actomyosin solution is compressed into bands. The contraction chamber is at the right. The torsion balance and its elongated arm are also shown. As the protein band contracts, the arm of the torsion balance moves with the contraction; the trough moves in an opposite direction. Thus, the counterforce produced equalizes the tension on the bands. (From Kako and Bing.<sup>4</sup>)

ished contractility on a molecular level, but the actomyosin bands have an advantage over actomyosin solutions in that studies on the essential property of the contractile proteins, their contractility, can be carried out. The results on actomyosin bands prepared from human hearts are in agreement with those of Benson, who, using glycerinated heart muscle strips from failing myocardium of dogs, found that these fibers did less work than fiber bundles from normal hearts under equivalent conditions of length, temperature, pH, and ATP concentration.<sup>10</sup>

We have asked ourselves repeatedly about what factors could cause a change in contractile elements of heart muscle leading to diminished contractility. We favored the hypothesis of stretch as playing an important role, but this has never been conclusively demonstrated. A change in orientation of actomyosin is also unlikely, since Olson observed changes in myosin molecules of failing heart muscle.<sup>6</sup>

Recently, a very interesting observation on failing hearts has been published by Meerson and Zayats.<sup>11</sup> Their report is of particular

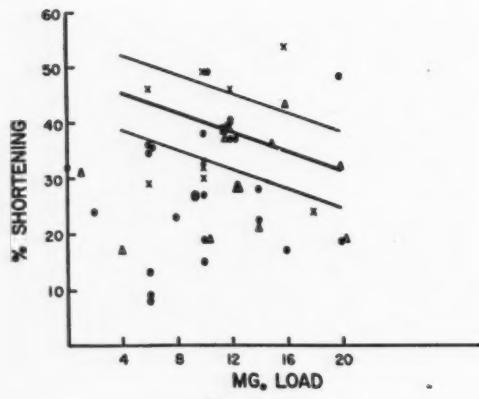


Figure 4

The contractility of actomyosin bands prepared from failing human hearts and the effect of digoxin and of calcium and digoxin combined. The regression line and standard deviations obtained from normal data are represented. Many of the points obtained from failing hearts are below the standard deviation of the normal. Digoxin does not influence the percentage shortening (the group mean is still below the deviation of normal data). The combination of digoxin and calcium results in marked improvement of contractility, indicated by the fact that the group mean is now on the normal regression line.  $\square$  = Control:  $b = -0.879$ ,  $p < 0.01$ ;  $\bullet$  = failure, control:  $b = -0.019$ ,  $p > 0.9$ ;  $\Delta$  = digoxin:  $b = 0.295$ ,  $p > 0.1$ ;  $\times$  = digoxin + Ca:  $b = 0.404$ ,  $p > 0.5$  (From Kako and Bing.<sup>4</sup>)

interest, since it touches on the previous discussion, while leading into the regulation of cardiac function by biochemical processes. These authors measured the rate of protein synthesis in hearts of rabbits with experimentally produced aortic stenosis.<sup>11</sup> Protein synthesis was determined by the rate of uptake of  $S^{35}$ -labeled methionine into the heart muscle. The changes occurring in protein synthesis during the development of failure are illustrated in figure 5. Immediately after the production of aortic stenosis, a period called the "state of sudden overload," the heart dilates and its weight increases. The rate of protein synthesis doubles and microscopic changes in heart muscle are noticeable. Muscle glycogen and creatine phosphate diminish, while lactic acid concentration in heart muscle rises. During the second stage, that of

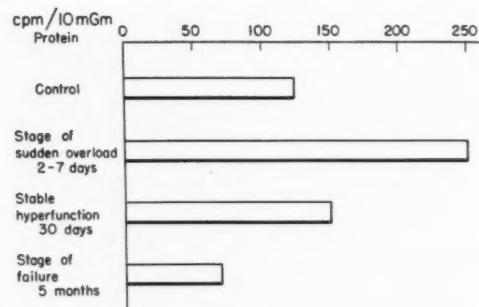


Figure 5

Data from Meerson and Zayats<sup>11</sup> are graphically illustrated in this figure. The rate of incorporation of  $S^{35}$  methionine into myocardial protein increases immediately after the production of aortic insufficiency and then decreases during the state of stable hyperfunction; it reaches its lowest level during myocardial failure.

stable hyperfunction, the heart weight first increases, then remains constant, and the rate of protein synthesis returns to normal. There is hypertrophy of the muscle fibers. The myocardial concentration of phosphocreatine and glycogen is normal, but the lactic acid concentration remains elevated. During the third stage, that of cardiac decompensation, the heart weight remains stable, but there is dilatation and protein synthesis decreases. (fig. 5). Lactic acid concentration in heart muscle increases, creatine phosphate diminishes, while glycogen concentration remains unchanged. Accordingly, cardiac hypertrophy produces an increased myocardial mass, and an increase in sarcosomes. Myocardial anoxia is present, as illustrated by the increase in lactic acid. The authors conclude that the disturbance in protein synthesis in the myocardium is an important factor in the development of myocardial failure and that the loss of kinetic energy of cardiac contraction is connected with a disturbance of the normal process of protein synthesis in heart muscle. The cause for diminished protein synthesis may be prolonged anoxia with reduced ATP synthesis or a deficiency of deoxyribonucleic acid (DNA), the latter brought about by a relative increase in the size of the cytoplasm as compared to nuclear mass.<sup>11</sup>

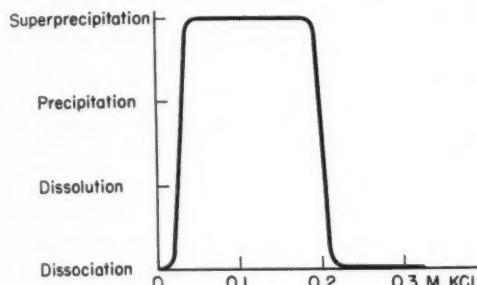


Figure 6

At very low potassium chloride concentrations and in the presence of ATP, actomyosin is dissociated. As the potassium chloride concentration is increased, superprecipitation occurs. As the ionic strength is further increased, dissolution and dissociation again take place. (After Szent-Györgyi.<sup>1</sup>)

#### The Role Played by Biochemical Processes in the Regulation of Cardiac Function

The challenging concept of Meerson and Zayats relating possible alterations in contractile elements to protein synthesis leads us into the second portion of the discussion, a consideration of biochemical events concerned with the functional regulation of the heart muscle. It is not difficult to predict the influence of these biochemical factors. They are likely to be responsible for a greater speed of contraction and should play a predominant role in the adaptation of the heart muscle to rapid changes in internal environment. Actomyosin bands, gels, superprecipitations have with few exceptions no ways of grading their responses except in an all-or-none fashion. Superprecipitation is an example. As mentioned before, in the presence of ATP, a slight increase in KCl causes an intense precipitation, provided one starts at low potassium chloride concentrations. If 2 consecutive test tubes differ by no more than 0.02 molar potassium chloride, dissolution is found in 1, superprecipitation in the other (fig. 6). In all likelihood, it is the presence of the cell membrane that is responsible for a smooth regulation of the intracellular ionic concentration. In addition to the presence of a membrane or membranes, the organelles of

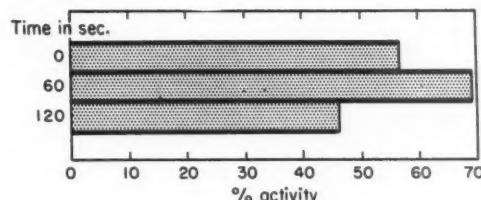


Figure 7

The effect of ventricular fibrillation on active phosphorylase activity of the left ventricle is shown. Active phosphorylase activity first increases, then diminishes, as ventricular fibrillation proceeds. Mean value of 4 experiments.

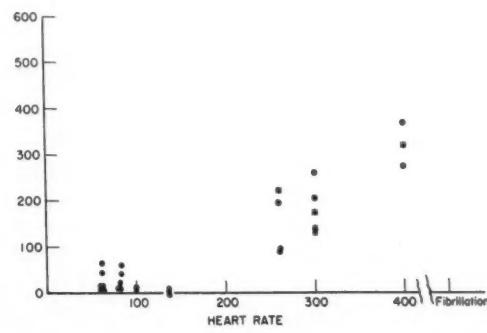


Figure 8

The rise in heart rate results in an increase in lactate concentration ( $\mu\text{M}/100 \text{ Gm. muscle}$ ) of left ventricular muscle. The highest concentrations are found in ventricular fibrillation.

energy production, the mitochondria regulate the speed of contraction *in vivo*. Their ubiquitous presence makes ATP accessible to every portion of the fibril, while in artificial models the contraction depends on the penetration of ATP from the bath solution.

Therefore, although actomyosin bands may present true models of the contractile elements, they are stereotyped and devoid of the ability to respond quickly and to adapt themselves independently to alterations in environment.

The relationship between enzymatic activity at the cellular level of organization and the functional activity of the heart muscle may be investigated by integrating changes in either the rhythm or the force of con-

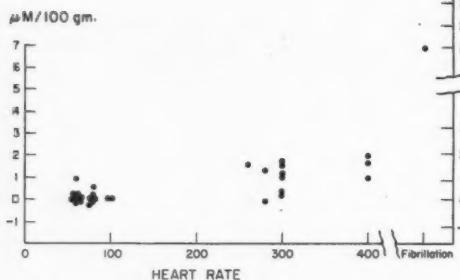


Figure 9

*Increase in the heart rate is accompanied by a rise in the glucose-6-phosphate concentration of left ventricular muscle. The highest values are found in ventricular fibrillation.*

traction of the heart with well-defined biochemical reactions. In 1943, the Coris discovered the enzyme phosphorylase, which catalyzes the reaction glycogen + inorganic phosphate = glucose-1-phosphate.<sup>12</sup> The enzyme exists in an active and in an inactive form; enzymes in muscle can convert the active into the inactive form or can catalyze the reverse reaction.<sup>12</sup> Phosphorylase appears, therefore, as an important enzyme in determining the rate of glycogenolysis in skeletal muscle. Cori, summarizing the effect of stimulation on phosphorylase a content of skeletal muscle, stated that increasing the speed, as well as the total number of contractions, causes a progressively greater increase in the amount of active phosphorylase; however, during tetanic contractions, the ratio of phosphorylase a to total phosphorylase diminished.<sup>12</sup>

An increased rate of stimulation of skeletal muscle also results in major changes in the carbohydrate intermediaries of the Embden-Meyerhof cycle. Thus, in anaerobic muscle, glycogen disappears, while lactate and hexosephosphate accumulates; apparently, phosphofructokinase becomes the rate-limiting step during anaerobic contraction.<sup>12</sup> If the response of the heart muscle and of skeletal muscle is similar, then the increased heart rate should result in glycogenolysis, with accumulation of glucose-6-phosphate (G-6-P) and lactic acid; it should also lead to an in-

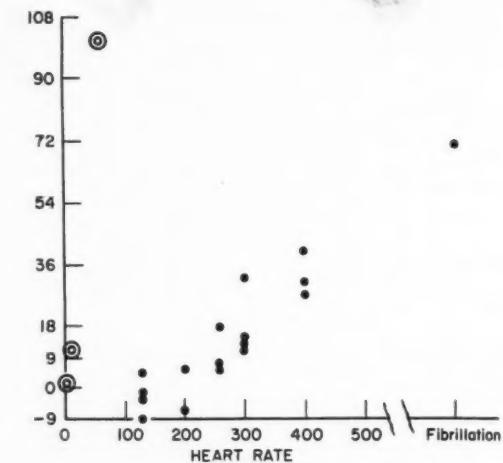


Figure 10

*Increase in the heart rate leads to a diminution in myocardial glycogen concentration during first 2 minutes (mg./100 Gm. muscle). As compared to skeletal muscle, glycolysis occurs at much faster rates of contraction. ◎=Skeletal muscle (Cori); ●=heart muscle.*

crease in the relative concentration of phosphorylase a to total phosphorylase. This supposition is correct but applies only to the anoxic heart muscle.

Figure 7 illustrates that ventricular tachycardia, with beats over 300 per minute, and ventricular fibrillation first cause an increase and then a diminution in phosphorylase a activity.<sup>13</sup> Similar results are obtained in atrial muscle during atrial fibrillation.<sup>13</sup> The increase followed by the fall in active phosphorylase can be explained by assuming that, during the first seconds of tachycardia, the heart muscle is still alkaline, favoring the enzyme that synthesizes phosphorylase a.<sup>12</sup>

The increased heart rate is also accompanied by definite changes in carbohydrate intermediates.<sup>13</sup> As in skeletal muscle, the increased rate of contraction leads to an increase in lactate and G-6-P concentration and to a decline in glycogen (figs. 8, 9, and 10). This suggests that, as in skeletal muscle, the enzyme phosphofructokinase is the rate-limiting enzyme under these conditions. Undoubtedly, anoxia as initiated by a decline in coronary blood flow is present under these

circumstances; this is confirmed by a more negative oxidation-reduction potential as calculated from the ratio lactate to pyruvate.<sup>12</sup>

The shift to an anaerobic metabolism and to glycogenolysis and to the transient increase in phosphorylase activity are the results of quick adaptive reactions in energy production; the actomyosin band or thread lacks this ability; it is the fly-wheel of the steam engine without the steam generator.

In the absence of myocardial anoxia, an increased rate of stimulation of the heart produces no alterations in either the concentration of carbohydrate intermediates or in the phosphorylase activity (table 1).<sup>13</sup> These results were obtained in hearts *in situ* in which coronary arteries were perfused from a donor animal. Apparently, the increased rate of stimulation, in the absence of anoxia, fails to evoke the metabolic pattern described as typical for muscular contraction. An increase in heart rate alone is not sufficient to stimulate the activated enzyme.<sup>13</sup>

Likewise, the increased force of contraction, for example, as initiated by angiotensin, is without effect on phosphorylase activity.<sup>13</sup> This enzyme therefore appears to be activated only under the influence of anoxia or catecholamines, which has been shown by Meyer and Moran and by Kukovetz and others.<sup>14, 15</sup>

The conclusions that may be drawn from this discussion are of a specific and of a general nature. In the first place, whatever the underlying physical chemical reasons may be, actomyosin prepared from failing human heart possesses diminished contractility. This adds some weight to the argument that the fundamental defect in heart failure lies in the organs of energy utilization. It is intriguing to consider a causal relationship between defective protein synthesis and diminished contractility.

In general, alterations in function of the heart that come into play upon rapid changes in demand are the result of the integration of several diverse biochemical reactions in the

Table 1  
Percent Changes in Phosphorylase A as a Result of Atrial Fibrillation and of Ventricular Fibrillation after Perfusion of the Coronary Arteries

Active phosphorylase % of total				Active phosphorylase % of total			
Time	Control* 3 min. after perfusion	Atrial fibrillation† 0.5 min.	Time	Control before perfusion	Control 3 min. after perfusion	Ventricular fibrillation 0.5 min.	
Exp.			Exp.				
13	22	21	17	22	22	20	
14	46	44	18	31	31	29	
15	28	31	19	37	37	38	
16	42	46	20	40	43	38	
Mean	34.5	35.5	Mean	32.5	33.3	31.3	
± S.E.	± 5.7	± 5.9	± S.E.	± 4.0	± 4.5	± 4.3	

\*Left atrial appendage.

†Right atrial appendage.

cell, which are regulated by the cell membrane and the substructures of the cell concerned with energy production. The contractile proteins are but following the lead and command of those cellular elements concerned with energy production and those endowed with the regulation of ionic transfer into, and out of, the cell.

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## Discussion

*Chairman Eichna:* Dr. Konigsberg, does ATP cause contraction of these early cells, either in the mononucleated phase or in the 3- or 4-day stage when cross striations appear?

*Dr. Konigsberg:* The multinuclear ribbons begin to contract spontaneously at about the seventh day of culture. By this time, cross striation is apparent. These contractions occur, however, without the addition of exogenous ATP.

We have added ATP to the glycerol-extracted cultures and these respond as one would expect of glycerol-extracted models; upon the addition of ATP they contract. You start out with a culture that is about the size of a quarter and get a dime in change.

*Dr. Mommaerts:* Dr. Konigsberg, it struck me that some of the effects of nitrogen mustard persist over several days; yet they leave a substance in water that persists only for about 15 minutes. Is the implication that the cells are influenced during this short time and that this influence persists?

*Dr. Konigsberg:* Yes, exactly. We prepare the mustard solutions immediately before use and expose the cells for an hour, although we realize that this may be longer than necessary. The first 15 minutes, as you point out, would probably be sufficient. We then replace the nitrogen-mustard solution with complete growth medium. What we measure subsequently is the damage that was done during those first 15 minutes.

*Dr. DeHaan:* Dr. Konigsberg, have you ever redissociated the syncytia before striations are formed from the glass and will they form single cell suspensions again?

*Dr. Konigsberg:* The difficulty we have experienced is that, upon the addition of trypsin to differentiated cultures, we get clumps of interwoven multinucleated ribbons in addition to free cells. These clumps have thus far resisted dispersion. If we remove the clumps by filtering the cell suspension through bolting silk and plate the free cells, we again get the formation of multinuclear cells—although

they are sparse. Several repetitions of this procedure eventually yield muscle-free fibroblast cultures.

*Dr. DeHaan:* Well, then, do you get the impression that these syncytia, once they form, do not disaggregate into single cells?

*Dr. Konigsberg:* No, I don't think so. We are not convinced that trypsin disaggregates the multinuclear ribbons, although Rinaldini has suggested that it does (*Exper. Cell Res.* 16: 477, 1959). Godman and Murray (*Proc. Soc. Exper. Biol. & Med.* 84: 668, 1953) applied colchicine and noted that the multinucleated cells broke up into smaller fragments that later re-associated. DeRényi and Hogue (*Arch. exper. Zellforsch.* 16: 167, 1934) observed contracting multinuclear cells that gave off mononucleated buds during this period of contraction. We think that a similar breakdown into mononucleated elements occurs during the early stages of muscle regeneration. The multinucleated muscle cell may represent a biologic metastable state that is completely reversible under the appropriate conditions.

*Dr. Fishman:* Dr. Konigsberg, would you be willing to say if you think that these observations also apply to cardiac muscle? Have these experiments been done on cardiac muscle?

*Dr. Konigsberg:* My former collaborator, Dr. William Cooper, is studying cardiac muscle. I think that he will probably get very similar results. One question of interest is whether there is a stage in developing cardiac muscle corresponding to that of developing skeletal muscle in which rows of contiguous nuclei form. I would suspect that this arrangement wouldn't occur in cardiac muscle because it isn't a true syncytium. The intercalated disc, as I understand it, is an actual physical separation between what were originally individual cells.

*Dr. Richard L. Klein* (Jackson, Mississippi): Do you see intercalated discs in embryonic cultures?

**Dr. Konigsberg:** We have worked only with skeletal muscle. I don't know.\*

**Dr. Klein (Jackson, Mississippi):** Dr. DeHaan, in view of your proposal of 2 distinct cell types in cardiogenesis, you are undoubtedly familiar with the work of Fänge, Persson, and Tesleff. Would you care to comment on the fact that myocardial cells, when cultured in tissue culture after trypanization, apparently will show a typical pacemaker type of potential up to 8 days?

**Dr. DeHaan:** Yes, I am familiar with their work<sup>38†</sup> and it is very interesting. I'm glad you asked this question, because it gives me an opportunity to expand on the apparent lability of pacemaker function in immature myocardium. As you've suggested, clusters of embryonic ventricular cells, when isolated in tissue culture, pulsate spontaneously and exhibit pacemaker prepotentials. Recordings taken from such clusters from 4-day hearts always show diastolic depolarization.<sup>38</sup> In contrast, Meda and Ferroni<sup>61</sup> have demonstrated that similar recordings from intact embryonic ventricle *in situ* do not show pacemaker prepotentials. Finally, Fänge, Persson, and Thesleff<sup>39</sup> demonstrated that cell clusters from 8-day ventricle sometimes, but not always, exhibit pacemaker prepotentials. Thus, it would seem that, in early stages of development, cells capable of functioning as pacemakers are widespread throughout the heart, but that in the normal course of development their spontaneous activity is suppressed or masked. When subjected to culture conditions, they once again exhibit prepotentials. Later in development, that is, at 8 days, it would appear that some cells have now been "fixed" as ventricular myocardial units and have thereby lost their ability to exhibit prepotentials, even under culture conditions. This is the only rea-

sonable explanation that I can think of at the moment.

**Dr. Mommaerts:** May I return to the high glycogen again?—because that is still very mysterious. You indicate, of course, that this high glycogen makes one think of glycolytic metabolism. But to explain it, i.e., the high content, we need a second fact, that is, some form of periodic use, because glycogen is a storage food, and we cannot understand its function unless there are periods in which it is used and other periods in which it is resynthesized. For example, by analogy, we find starch deposited in the roots of trees during the "fat" season and then used during the "lean spring" when leaves have to grow. Later on, starch is replaced. Unless we find similar periodicity in the use of glycogen in the heart, we will be unable to understand why it is there.

**Dr. DeHaan:** This is a most interesting topic, which I have not worked on myself. However, to add fuel to your argument, Schiebler<sup>17</sup> states that the glycogen in the conduction system is more resistant to a period of anoxia than is glycogen in the myocardium; that is, even under prolonged periods of anoxia, glycogen disappears more slowly from the conduction fibers than from the ventricular muscle. On the other hand, the enzymologic information that I referred to earlier suggests that conduction tissue has a relatively low rate of oxidative metabolism. These are the observations as they now appear in the literature. I'm afraid I can't go beyond them.

**Dr. Rhodin:** I should like to hear a comment about the ability of the conducting system to contract. Do you know of any studies in which the conductive cells have been raised in pure culture?

**Dr. DeHaan:** No. To my knowledge, the culture of conductive cells has not been done. I am, in fact, at present getting organized to do tissue culture and have considered attempting just this project. There are statements in the literature to the effect that the bundle and bundle branches of some species, when dissected out, are relatively noncontractile,

\*Since this symposium was held, a note has appeared describing the development of cardiac muscle cells from disaggregated cell suspensions (*Science* 132: 1839, 1960).

†References in Dr. DeHaan's discussion identified by superior number will be found in "References" at the end of his paper.

even when stimulated. Perhaps Professor Weidmann would comment on this.

**Dr. Weidmann:** Excised Purkinje fibers of ungulates (sheep, calf, horse) usually do not contract, even when looked at through the microscope, although they may generate, and will always propagate, action potentials. However, by increasing the calcium concentration in the bathing fluid 2- or 3-fold, an action potential will invariably be associated with a visible contraction. This indicates that even the most differentiated conducting tissues have retained the potential for exciting a mechanical response under suitable conditions.

**Dr. Arthur H. Briggs:** Dr. Olson, from your studies of cardiac myosin in congestive heart failure, do you believe that your animals are in a stage of irreversible failure and whether any new insights could be gained by giving digitalis or, perhaps, by releasing some of the pulmonic stenosis?

**Dr. Olson:** In general, we have not been able fully to reverse this failure either by sodium restriction, or by the use of diuretics or of digitalis. We have done a few preliminary studies on the effect of digitalis upon myosin isolated from the hearts of both normal animals and animals in failure. We have shown that digitalis is bound to myosin (*Fed. Proc.* 18: 221, 1959). Although the effects on the protein are variable, there is one consistent feature, that is, the digitalis tends to make myosin more unstable. This may have some implications for our hypothesis. If, in fact, trimers are not so well utilized in the contractile mechanism as monomers, something that would produce even disorganized fragments of the polymerized myosin might conceivably be beneficial to the subject with heart failure.

Another point is that, with heart failure resulting from tricuspid insufficiency and pulmonic stenosis, we do not have a good model to test the effects of inotropic agents upon sodium retention. Stimulation of the right ventricle to greater contractility with digitalis usually further elevates the right atrial pressure and may lead to further sodium retention. We are going to restudy this problem in animals with primary left heart

failure, which should give a better answer to your question and hopefully a clearer picture of the effects of digitalis upon myosin.

**Dr. Ellis S. Benson** (Minneapolis, Minn.): One has a natural desire to defend one's work to the hilt, but there is also the need to be objective. Dr. Olson has referred to our work on actomyosin and on glycerol-extracted trabecular-muscle preparations from the chronically failing heart. In both of these preparations one must realize that we are dealing with a rather unstable, poorly characterized preparation subject to much inherent variability.

Dr. Olson and Dr. Bing present an interesting case for the existence of changes in the myocardial contractile proteins in chronic congestive heart failure. I think that, on the basis of our experience in this field, I would urge an attitude of caution in drawing conclusions, pending further investigation.

**Dr. Bing:** Dr. Huxley, based on your work on the sliding in and out of actin and myosin fibers, what do you believe is happening during prolonged stretch? After all, the failing heart is stretched for a long time. What happens during chronic stretch?

**Dr. Huxley:** I think that the degree of overlap of the filaments would obviously be less when a muscle is working in prolonged stretch. In the particular model that I am supporting, I see no reason to suggest that the prolonged stretch would be detrimental to the muscle. Beyond that, I wouldn't say anything.

Does anyone know the amount of myosin per gram of muscle in these failing hearts?

**Dr. Olson:** We haven't studied this in a really quantitative way, because our objective was to obtain a purified sample for characterization. From the size of the precipitates at various stages of purification, however, I would say that the difference between the normal and the failing heart with regard to myosin is not marked.

Is there any evidence, Dr. Huxley, that in chronic stretch the A-band changes at all?

**Dr. Huxley:** As I say, I haven't looked at muscle in chronic stretch.

**Dr. Olson:** I ask this question because it

seems possible to me that some degree of depolymerization inside the A-band may be a feature of a "normal" heart. This depolymerization could be a feature of the organization of the protein in the thick filament, terminating in the double strands of myosin which protrude and show as "feet." If there were stresses that might distort the A-band filament, which, I think, we have to accept now as a sort of organelle, this distortion, in turn, might cause internal changes in the state of organization of the protein itself and result in polymerization. This is the kind of idea we have been speculating about.

*Dr. Huxley:* Is there any effect of chronic stretch on any other muscle than heart muscle?

*Dr. Olson:* We are in the process of studying that now in the rabbit and the dog. We are trying to extend the femur in such a way that we can get a chronically stretched leg muscle for study. It would be interesting if we could generalize the hypothesis of polymerization with chronic stretch to skeletal muscles.

*Dr. Mommaerts:* Before coming to my question, I must express doubt that chronic stretch of skeletal muscle occurs under biologic circumstances; even if you put people on the rack, you are only stretching their ligaments, aren't you?

*Dr. Olson:* This is the reason why we have to rely on the surgeon again. The gross difference between cardiac and skeletal muscle in the intact organism is that the skeletal muscle has an origin, an insertion, and a fairly fixed range of motion.

*Dr. Mommaerts:* To start with a small technicality: the molecular weight of skeletal myosin in our preliminary paper was 380,000, but, in the final paper, when we applied a correction for concentration dependence of light scattering, the weight increased to 420,000 plus. With the exception of one recent paper by Harrington and Kielley (*Biochim. et biophys. acta* 41: 401, 1950), where it has gone up higher, this value of 420,000 to 450,000 is now being found by just about everybody, both as a molecular weight and also as a bind-

ing equivalent for 1 molecule of ATP. This discrepancy will have to be explained.

I can only say that the data that Dr. Olson has presented look completely convincing. There has, of course, been controversy on this point, but what we have seen here leaves no place for any other conclusion but that there is a different myosin in the failing and in the normal heart.

Now as to his concept as well as that of Dr. Bing—that this difference in myosins characterizes heart failure. They are saying something very different from what I said yesterday when I stated that heart failure is a disease of the force-velocity relation. But this relation must have a mechanism; and, in chronic cases, this relation could very well be along the lines suggested by Dr. Olson. In other words, what sounded so different yesterday is only a difference of emphasis and not, in any fashion, a contradiction of the concept that we have just heard.

*Dr. Olson:* I should like to add 1 point about the findings of Harrington and Kielley which give a molecular weight for skeletal myosin of about 620,000. Again, this could be an artifact of preparation. I don't think it is an artifact of physical chemistry. With guanidine salts, Harrington and Kielley have been able to depolymerize their skeletal myosin into a particle of 216,000 molecular weight, which in particle size matches very closely the cardiac myosin that we have isolated from the heart, although the physical constants indicate that it is more unfolded. It could be that their particle is the fundamental particle in the myosin series, of the order of 220,000 in molecular weight. In vivo, this particle could give rise to molecules with different degrees of polymerization. Some such polymers could be formed under "pathologic conditions." We know that even more extensive polymerization can occur *in vitro* as a result of denaturation.

*Question from Audience:* Dr. Olson, in your slide summarizing the differences between myosin obtained from normal and failing hearts, you included a statement for ATPase activity; you stated that this was not

a significant difference, although it appeared to be significant from here. Would you comment on this?

**Dr. Olson:** The question was, what about the ATPase values for the normal and failing heart? Actually, the range of activity that has been obtained in the study of the normal and failing heart is such that a statistical analysis of the 2 groups of values shows no significant difference. It would thus appear that the active site for ATPase is not altered per unit weight, which one might assume is reasonable if the polymerization does not involve linkages around the active site.

Perhaps a more important fact is that skeletal myosin has a much higher ATPase activity. That has been found consistently and is also true of the heavy myosin fragments after tryptic digestion.

Another point of difference is that we really cannot find heavy myosin fragments in our products of tryptic digestion of cardiac myosin.

**Dr. John Gergely** (Boston, Massachusetts): Dr. Olson has made an excellent case for both the low molecular weight of normal heart myosin and the high molecular weight of myosin obtained from failing hearts. However, I find it rather difficult to set aside the results of Davis and his colleagues (*J. Clin. Invest.* 39: 1463, 1960) at the National Institutes of Health, and also those of our laboratory (Gergely, J., and Kohler, H.: *Fed. Proc.* 16: 185, 1957), which consistently indicate a molecular weight of normal heart myosin of around 450,000. I should add that, since we used the light-scattering technic, which was preceded by extensive centrifugation at high dilution, I cannot quite see how our preparations could have been contaminated with actomyosin.

However, setting this aside, I should like to make a constructive suggestion, putting two and two together: that is, putting together what Dr. Mommaerts said in one of his comments about their extremely ingenious method of measuring ATP binding, and the ideas of various degrees of polymerization that Dr. Olson has brought up.

If Dr. Olson is right, then one should find that in heart myosin the combining weight of myosin with ATP is about 200,000, and this would not be affected by the presence of any small actomyosin impurities or aggregation. We have made some measurements on the binding of pyrophosphate (which can serve as model substance) to heart myosin, and we found that 1 mole of pyrophosphate combined with about 500,000 Gm. of protein. Perhaps Dr. Mommaerts would do these experiments and, by his technic, we could have a quick and decisive answer. Naturally, the fact that the combining weight for ATP of skeletal myosin is about 400,000 to 500,000 would seem to rule out a simple dimer picture for the skeletal protein.

**Dr. Olson:** We have thought about these studies. Perhaps Dr. Mommaerts and I can collaborate in applying the firefly technic to the study of this binding constant for normal cardiac myosin.

The problem of a difference in molecular weights obtained for cardiac myosin in different laboratories may be physiologic rather than physicochemical. We have observed, for example, that, in normal animals allowed to suffer anoxia during the period of sacrifice, polymerization of myosin occurs, and higher particle weights are obtained in normal animals. If one allows time to elapse before the heart is chilled in ice water at 1 degree, one finds changes that affect particle size; in other words, there seems to be a very labile system for post-mortem polymerization. This should be explored further.

**Question from Audience:** Could Dr. Olson comment further about the time element with reference to the human studies, where studies of heart myosin are done 2 or 4 hours post mortem?

**Dr. Olson:** We all appreciate that it is exceedingly difficult to obtain human cardiac muscle shortly after death. It is possible, as Dr. Bing has shown, that the actomyosin fibril, no matter how it is composed *in vitro*, will contract if isolated any time up to 6 hours after death. What the size of the myosin monomer is under these conditions, of course,

is unknown. I would doubt that it remains unchanged for 6 hours post mortem. Nevertheless, we are going to try to study human cardiac myosin under various conditions. Possibly we can control the viability of the preparation by plotting a curve of mitochondrial phosphorylation, or some other index, as evidence for the intactness of the muscle and extrapolate to zero time.

**Dr. Furchtgott:** I should like to comment again on this matter of whether there is a deficiency in the conservation of energy in the high energy phosphate pool. In my paper yesterday, you may remember, I commented on the work of Drs. Feinstein and Schwartz on the hearts of guinea pigs in chronic failure due to aortic stenosis. They found respectively, a fall in high energy phosphates, especially creatine phosphate, and about a 30 to 40 per cent uncoupling of oxidative phosphorylation. I was cautious about interpreting their results and did not conclude that the fall in efficiency of oxidative phosphorylation and levels of high energy phosphate produced the failure. These defects may have come about subsequent to a failure as a result of various other changes. But I should like to stress that, if such defects are associated with failure, they would certainly aggravate the situation, making for an even worse condition of the myocardium than would have existed in their absence.

I was interested to hear about the results of the Russian workers, which Dr. Bing reported. Apparently, in the later stage of failure, they, too, found a fall in creatine phosphate. Is that right?

**Dr. Bing:** You are correct. During the first stage (which they call the accident stage), when the heart dilates rapidly, Drs. Meerson and Zayats observed a fall in creatine phosphate, but no change in ATP. A second diminution in creatine phosphate and also a decrease in ATP occurred during the final stage, that of gradual decompensation.

**Dr. Furchtgott:** It may be equivalent to what Feinstein finds in the markedly hypertrophied hearts of guinea pigs in severe congestive failure.

**Dr. Bing:** I agree with Dr. Furchtgott that there are different types of mechanisms which lead to heart disease. Lack of high energy phosphate may be one of them. Other situations that may start the development of heart failure may be truly metabolic, such as occurs in beriberi heart disease.

**Dr. Olson:** I should just like to emphasize, Dr. Furchtgott, that, at least in our experimental preparation, there is no evidence in the state of advanced heart failure for any decrease in CP storage. The mitochondria in these animals seem normal *in vitro*. This is not to say that one can't get a mixed lesion, although I think, even in the guinea pig, there has been a report by Plaet and Gertler (*Ann. New York Acad. Sc.* 72: 515, 1958) that mitochondria from guinea pigs in failure from aortic stenosis do phosphorylate normally; so I think that it is possible that more studies of chronic animals may reveal a component of anoxia resulting from coronary ischemia. This is just a suggestion but I think it is very clear that one can have cardiac failure without a change in oxidative phosphorylation.

**Dr. Furchtgott:** I agree that you could have it. All I'm saying is that there may be examples of failure—even clinical failure—in which this is an additional aggravating factor. I'm not saying that it is the initial cause of the failure.

**Dr. Olson:** Yes. I have just 1 other comment about Dr. Bing's talk, if I may, with regard to the Russian work on incorporation of S<sup>35</sup>-methionine into total protein. An alteration in the contractile proteins and a change in S<sup>35</sup>-methionine uptake are not necessarily mutually exclusive phenomena. It may well be that this abnormal protein does not turn over so fast; in fact, we know that myofibrillar proteins turn over very slowly indeed.

Dreyfus, Kruh, and Schapira (*Biochem. J.* 75: 574, 1960) studied the uptake of C<sup>14</sup>-glycine into the skeletal myofibril in the rat and found the turnover to be remarkably slow. Further, the slope of the isotope-decay curve suggested that the myofibril is, like a red cell, undergoing little decay, but only dissolution. Further changes could occur in failure.

*Dr. Huxley:* I was wondering if anyone had tried to make heavy myosin from this low molecular weight, and, if so, what does its molecular weight come out to be?

*Dr. Olson:* We have tried it and failed.

*Dr. Hoffman:* I wonder if Dr. Bing would elaborate a little bit on the study reported from the Russians on the decreased incorporation of sulfate into protein? Is it possible that this change results in any way from the altered nutritional status of an animal that has had aortic stenosis for many months and presumably may have been near death at the time of the study?

Then, also, on his own data, I wonder if he would elaborate a little on the extent to which changes in phosphorylase activity are related either to anoxia or to the release of catecholamines? If it is primarily anoxia, how much anoxia, independent of catecholamine action, do you need to produce these changes?

*Dr. Bing:* Meerson and Zayats have excluded the possibility of the nutritional status of the animal as contributing to defective protein synthesis in heart muscle. This they have done by showing that the changes in protein synthesis in heart muscle are not paralleled by synthesis of plasma protein. As for Dr. Hoffman's second question, there appears little doubt that the changes in phosphorylase activity are related to anoxia. What role catecholamines play in this connection is not clear, since we have not performed any determinations of catecholamines under these circumstances.

*Dr. Paul F. Cranefield* (New York, N. Y.): If you do the same experiment that was mentioned, that of cross circulation, the heart, which is put into fibrillation, shows no deteriorating changes in its electric activity and the single-cell potential except for the changes resulting from the fact that the rate is very

high. The single-cell potential is not characterized by loss of resting potential, slurring of upstroke, or anything like that, which confirms the findings that deteriorative changes in fibrillation are linked simply to inadequate perfusions. Probably the only reason that electric defibrillation ever fails is that adequate oxygenation has not been maintained between the time of the initiation of fibrillation and the application of electric defibrillation. I wonder if anyone can suggest a way to distinguish between myocardial changes that arise from the mechanical load and those myocardial changes that come about as a result of the circulatory deficiencies of the myocardium. I do think that these may be 2 quite different phenomena. It would be nice if you could have a heart under the load of aortic stenosis but supplied with a normal coronary circulation. But, of course, that is impossible.

*Dr. Bing:* Dr. Cranefield, are there any changes in the resting potential in your anoxic heart-muscle cells?

*Dr. Cranefield:* No, you do not see the noticeable drop in resting potential which you do see if a heart is simply fibrillated without a maintained perfusion.

*Chairman Eichna:* I am obliged to call this discussion to a close. But before I subside, I should like to indicate that it has taken a great deal of restraint not to burst in on the discussion from time to time. As you depart, I would leave a few questions with you: Why does the trimer of myosin not contract as well as the monomer? How does the trimer work in with the Huxley demonstration of the location of the myosin? Are these trimers specific for the heart muscle or are they just functions of the malnutrition which these animals, including man, go through? I have many other questions which I shall reserve for private conversations.

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## V. Electrophysiology

Chairman: Chandler McC. Brooks, Ph.D.

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### Introduction

By Chandler McC. Brooks, Ph.D.

**I**N THE closing session of this symposium, we are to consider a function that actually precedes the mechanical contractile responses we have heard so much about. The heart beat originates in a pacemaker, where spontaneous depolarization occurs. Conduction of excitation involves a transmission of this depolarization over a specialized conducting system and then, ultimately, to the basic contractile fibers of the myocardium.

Dr. Weidmann, in starting the afternoon's meeting, will discuss membrane excitation in cardiac tissue. Dr. Hoffman will then describe the studies, which he and Dr. Paul Cranefield have carried out recently, on the conduction process in the specialized conducting tissue; in their paper, we shall be turning back to a matter that has been discussed at some length by previous speakers. I think that by

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the end of this symposium we shall see clearly that 3 or 4 major problems have been emphasized. One of them will be the problem of transmission in the conducting system and another will be the meaning of the intercalated disc.

Our third subject of the afternoon will bring us again to the matter of contraction; this topic will also involve a discussion of membrane phenomena.

The final presentation of the day will be devoted to a completely new subject. Dr. Hajdu will be concerned with materials that affect the contractile process. In brief, we will begin this afternoon with a new approach to the problem of cardiac function; then, as the afternoon progresses, we will reconsider topics that you heard discussion of yesterday and this morning. Our session will begin with Professor Silvio Weidmann of Berne, who will describe the processes involved in "Membrane Excitation."

### The Experimental Basis of Concepts

Einstein's theory of relativity, the most magnificent achievement of modern physics, was suggested by closest adherence to experimental facts; this is its strength. We may admire the grandeur of its structure of thought and the depth of its ideas; but this alone would never have secured for it that firm position in physics which it enjoys today. This position was secured because it is able to explain experimental facts, to foretell events; it was the later confirmation of these events which made this theory great.—H. Reichenbach. *From Copernicus to Einstein*. New York, Philosophical Library, 1942, pp. 51-52.

## Membrane Excitation in Cardiac Muscle

By SILVIO WEIDMANN, M.D.

The contributions made during the past 10 years are reviewed. Intracellular recording has made it possible to state absolute values for the cardiac resting potential (90 mv., inside negative to outside) and the "overshoot" during activity (30 mv., inside positive to outside). The surface membrane of a resting fiber is considered to be predominately permeable to K ions. During activity, Na conductance increases and K conductance decreases. The latter process is thought to be essential for explaining the high membrane resistance that is measured during the long-lasting "plateau" found with cardiac muscle. A hypothesis is presented that would account for the termination of the plateau and the beginning of repolarization.

IT IS THE PLAN of the present survey to begin with a description of the electrical events during cardiac activity; to continue with their interpretation in terms of the movements of ions; and to close by treating a more special problem: the possible reasons for the long-lasting action potential that is typical for cardiac muscle.

### Intracellular Recording

It was in 1949 that Ling and Gerard,<sup>1</sup> working in Chicago, described a new tool that stimulated electrophysiologic research in almost all its branches: the capillary microelectrode. Two people who had been taught the technic by Ling were to become responsible for extending the method from skeletal to cardiac muscle: J. W. Woodbury,<sup>2</sup> then working at Salt Lake City, and A. L. Hodgkin of Cambridge, England. I can well remember the day of July 16th, 1949. Having learned the microelectrode technic at Cambridge and having been rather unsuccessful in prodding around in different tissues of the frog, I became a heart physiologist by 2-fold chance: from 5 to 6 p.m. Dr. Feldberg had demonstrated a Starling preparation to the medical students and allowed me to cut out the dog's heart; and my wife agreed that I need not be home for supper at 6:30.

Now, what is the advantage of the new technic? The suction electrode, introduced

and widely used by Schütz,<sup>4</sup> had revealed the time course of the cardiac action potential with a fair amount of accuracy. Only by the use of the Ling-Gerard electrode, however, was it possible to record the full amount of the potential difference existing between the inside and the outside of a cardiac fiber.

Figure 1 illustrates the potential changes that can be observed when a microelectrode is introduced into a rhythmically driven preparation (sheep ventricle). The "zero line" or "reference potential" is first recorded between two *extracellular* electrodes (see drawing upper right). One of the electrodes then is moved into the preparation. Touching and penetrating the endocardial layer causes minor potential changes (first arrow). The penetration of a muscle fiber by the electrode tip (second arrow) is signaled by the occurrence of large potential changes synchronous with the contractions. In diastole the potential difference is constant (resting potential, here 85 mv.). On pulling back the electrode the potential drops to the reference level. When viewed on a faster time base (fig. 1, lower records), the transmembrane action potential shows an extremely rapid upstroke (1 msec.) followed by a plateau and a moderately rapid downstroke (repolarization).

The upstroke would coincide with the QRS complex and the downstroke with the T wave of a surface ECG.

### The Distribution of Ions

In heart muscle, as in other living cells, the ionic composition of the inside differs markedly

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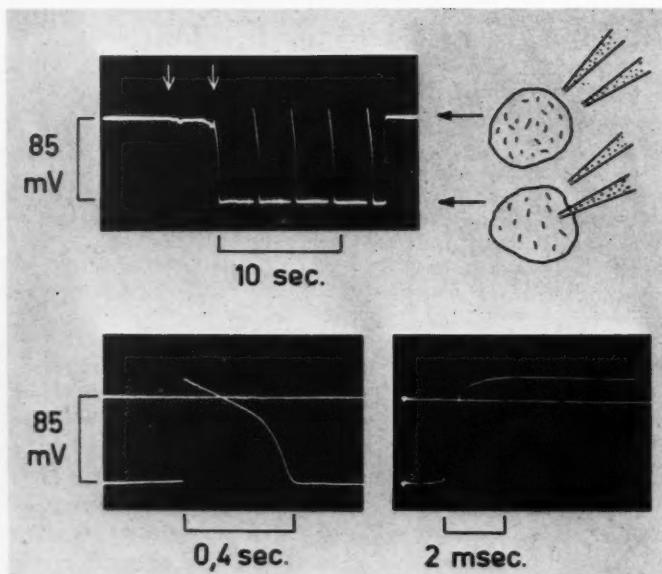


Figure 1

Upper record. Potential changes as observed during the introduction of a Ling-Gerard electrode into a myocardial fiber. The electrode positions corresponding to the "zero" and to the "trans-membrane" potentials are seen on the upper right. Lower records. From the same preparation, at higher sweep speeds, to bring out a single action potential (left) or its initial phase (right).

ly from that of the extracellular space. There are different views on how the inside composition is maintained in spite of the established fact that the membrane is permeable to all the ionic species that have to be considered (fig. 2). One of the simplest hypotheses is this: Na ions leave the cell by some "pump" process of an unknown nature. Na outflux takes place against an electrical gradient and a con-

centration gradient; for thermodynamic reasons it requires metabolic energy. K and Cl ions may be looked upon as being passively distributed. In the case of K ions the force from the concentration gradient (outward) would be balanced by the force from the electrical potential gradient (inward for a positive ion). It may be stated that the ratio of the K concentrations, 30:1 (inside:outside), and possibly also that of the Cl concentrations 1:30 (?) are in good agreement with the measured potential difference of about 90 mv. (for references see Weidmann<sup>5</sup> and Hoffman and Cranefield<sup>6</sup>).

#### The Movement of Ions

If the inner surface of a cell membrane is to become more positive, as during the upstroke of an action potential, the positive charge must be shifted from outside inward. During repolarization, on the other hand, the positive charge must leave the cell. Identification of the ionic species that carry the charge has been accomplished on a quantitative scale for the giant nerve fibers of the squid, mainly by the "Cambridge group."<sup>7</sup> Experiments on cardiac tissue then revealed many similarities (for references see Hoffman

#### Resting cell

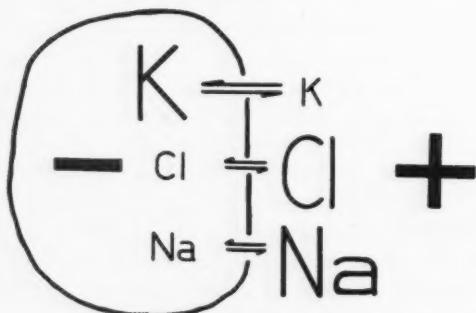


Figure 2

Distribution of ions between the inside and the outside of a muscle fiber. Exchange at rest for various ions is indicated with arrows of different length.

and Cranefield<sup>6</sup>). Thus it is generally agreed that the movements indicated by figure 3 are responsible for the cardiac action potential: inward movement of Na ions for depolarization, outward movement of K ions for repolarization. These shifts may be "passive," that is, they may be brought about by a succession of permeability changes: first a transient increase of Na permeability, then a slight increase of K permeability.

The state of ionic concentration differences represents stored energy and makes it possible for strong ionic currents to flow during the action potential. At the end of activity, the inside of the fibers will have gained a minute quantity of Na ions and lost a similar quantity of K ions. If ionic order is to be maintained over a longer period, Na ions must be ejected and K ions accumulated during the time between the two action potentials.

#### Evidence for an Increase in Na Permeability During the Action Potential

Since the work of Overton<sup>8</sup> it has been known that Na ions are necessary for cardiac excitation. Figure 4\* shows an experiment of the type that led to the conclusions expressed in the preceding section. Replacement of 80 per cent of the normal NaCl by choline chloride—choline being a nonpenetrating ion—has the following effects: (a) the resting potential remains unaltered, suggesting that the resting membrane is but sparingly permeable to Na ions; (b) the amplitude of the action potential and its duration decrease, suggesting that normally there is an inward Na current during activity. Furthermore, the upstroke velocity of the action potential can be shown to be roughly proportional to the extracellular Na concentration<sup>9, 10</sup> suggesting that during the initial phase of activity Na ions are the main carriers of charge.

#### The Plateau of the Cardiac Action Potential

In a nerve fiber, the action potential is ended in less than a millisecond; in the mammalian ventricle, activity lasts for a few tenths

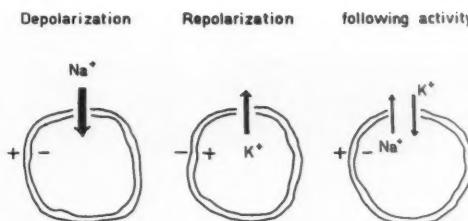


Figure 3

The shifts of ions leading to the changes of the membrane potential during electrical activity. Restitution of ionic gradients after activity.

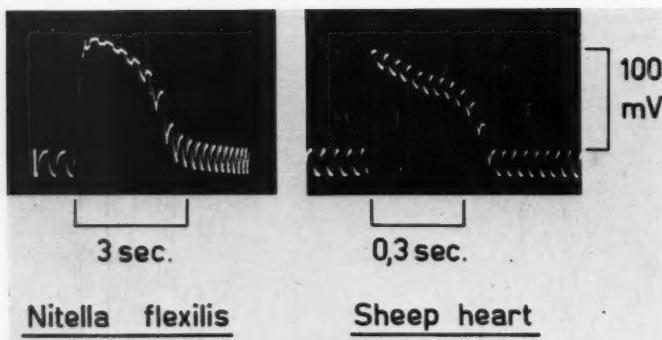


Figure 4

Evidence in favor of low Na permeability at rest and high Na permeability during activity. Successive action potentials of the same fiber of sheep ventricle. The vascular bed of the preparation was perfused first with normal Tyrode solution, then with a test solution containing 1/5 of the normal sodium. The camera was opened during the first 30 seconds after the admission of the Na-poor solution, then again between 60 and 90 seconds. (From Délèze.<sup>9</sup>)

of a second. During the plateau phase there is but little change in the membrane potential as a function of time, a state which means that inward current is almost equal to outward current. A further analysis will be facilitated if more is known about the membrane resistance. Figure 5 shows (left) an experiment performed on a sweet-water alga kept in Upsala tap water (method of resistance measurements<sup>11</sup>). During the plateau of its long-lasting action potential, the resistance is low; and a detailed analysis<sup>12</sup> of data from the alga *Chara* has revealed that during that phase a strong potassium inward current is balanced by a strong chloride outward current. With mammalian ventricular fibers, the

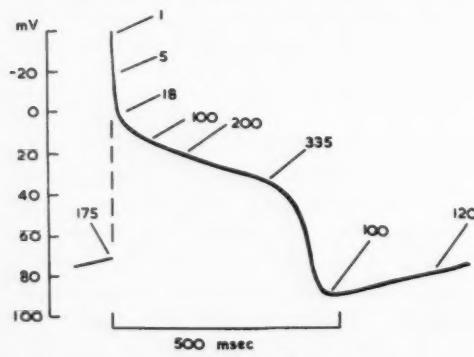
\*Figure 4 reproduced from Délèze: Circulation Research 7: 461, 1959. By permission of the American Heart Association, Inc.

**Figure 5**

Measurement of membrane resistance during activity. Square pulses of hyperpolarizing current were applied to the preparation. The resulting voltage change is a measure of resistance. Left: With *Nitella flexilis*, a sweet-water alga, low resistance on "plateau." Right: With sheep myocardium, high resistance on "plateau."

resistance during the plateau is relatively high (fig. 5). This is an important finding, which indicates that the increase of the  $\text{Na}^+$  permeability causing the upstroke of the action potential is at least partially reversed, while the permeability to other ions remains constant or even decreases.

Data of a more quantitative nature have been obtained for Purkinje fibers of the sheep heart (fig. 6).\* The action potential of Purkinje fibers regularly shows an initial spike and thus a comparatively "low" plateau; as a rule there is some potential drop during diastole, which is typical for all membranes

**Figure 6**

Membrane resistance during activity of a sheep Purkinje fiber, for comparison with figure 9. Relative resistance values are indicated for different phases of the action potential. (From Weidmann.<sup>13</sup>)

\*Figures 6 and 10 reproduced from Weidmann: Ann. New York Acad. Sc. 65: 663, 1957.<sup>13</sup> By permission of the New York Academy of Sciences.

with pacemaker properties. The figures attached to the tracing indicate that the resistance is even higher during activity than at rest.

The behavior of the nerve membrane was adequately described by Hodgkin and Huxley<sup>7</sup> with the aid of a set of empirical equations. Briefly, it was assumed that depolarization causes a transitory increase of  $\text{Na}^+$  conductance ( $G_{\text{Na}}$ ) and a long-lasting but somewhat delayed increase of  $\text{K}^+$  conductance ( $G_{\text{K}}$ ). The increase of  $G_{\text{Na}}$  would be responsible for the inward current, causing depolarization; the decrease of  $G_{\text{Na}}$  with a simultaneous increase of  $G_{\text{K}}$  would provide the outward current, causing repolarization.

An attempt to "produce" a Purkinje-fiber action potential by applying the Hodgkin-Huxley equations was recently made by Noble<sup>14</sup> (fig. 7).\* In choosing appropriate parameters, the high membrane resistance of Purkinje fibers during the plateau could be simulated only if  $G_{\text{K}}$  was allowed to fall as a consequence of depolarization. The conductance changes computed by Noble's machine are seen in figure 8.\*  $G_{\text{Na}}$  rises as a consequence of depolarization; after the initial spike it settles down at about 8 times its resting value.  $G_{\text{K}}$  by contrast falls as the driving force for  $\text{K}^+$  outflux increases.

\*Figure 7 (left) reproduced from Draper and Weidmann: J. Physiol. 115: 74, 1951;<sup>10</sup> and figures 7 (right) and 8 from Noble: Nature 188: 495, 1960.<sup>14</sup> By permission of the Journal of Physiology and of Nature.

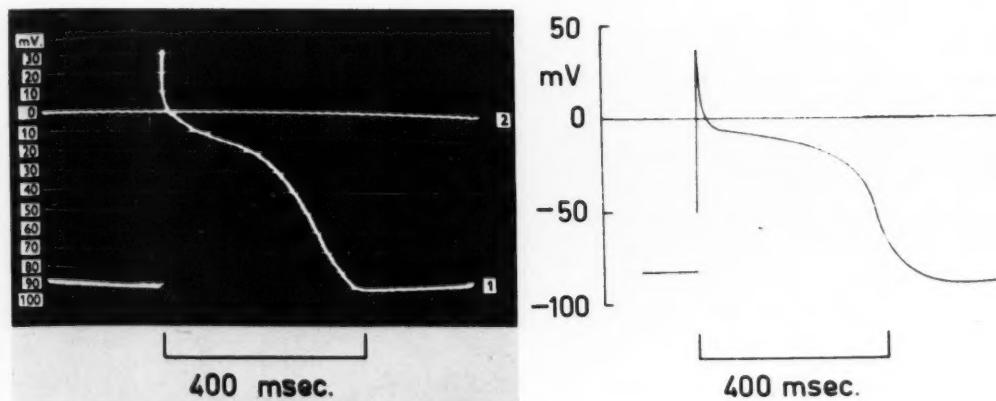


Figure 7

Left. Recorded action potential of a dog Purkinje fiber. (From Draper and Weidmann.<sup>10</sup>) Right. Similar action potential, computed with the aid of the Hodgkin-Huxley equations. (From Noble.<sup>14</sup>)

Convincing experimental evidence is available in the case of the nerve membrane to show that  $G_K$  rises as a consequence of depolarization.<sup>15</sup> It is important then to provide experimental evidence for the suggested drop of  $G_K$  in Purkinje fibers. With this intention, membrane resistance was measured over a large range of membrane potentials. To minimize the contribution of Na ions, the experiments were performed in choline chloride (Hutter and Noble;<sup>16</sup> Carmeliet<sup>17</sup>); to eliminate even chloride ions, as carriers of charge, a solution of choline acetylglycinate was used.<sup>17</sup> Under such experimental conditions, the membrane current practically has to be carried by K ions.

Figure 9 reveals that the membrane resistance corresponding to a membrane potential of -40 mV. (plateau level) is indeed 3 to 4 times higher than that corresponding to -90 mV. (resting level). This is taken to suggest that the assumption of a low  $G_K$  during the plateau is well justified.

#### The Changes Responsible for Repolarization

Applying long pulses of depolarizing current to a Purkinje fiber in a Na-free solution, Hutter and Noble<sup>16</sup> found a slow decrease of membrane resistance that was complete at the end of a few tenths of a second. This change

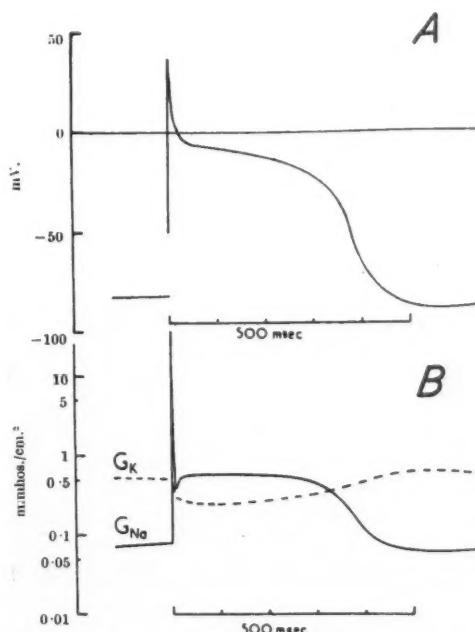


Figure 8

A. Computed action potential, same as in figure 7. The integration was started by displacing the membrane potential to -50 mV. B. Time course of membrane conductance plotted on a logarithmic scale.  $G_K$  denotes potassium conductance.  $G_{Na}$  sodium conductance. The potassium and sodium equilibrium potentials were set at -100 mV. and +40 mV. respectively. (From Noble.<sup>14</sup>)

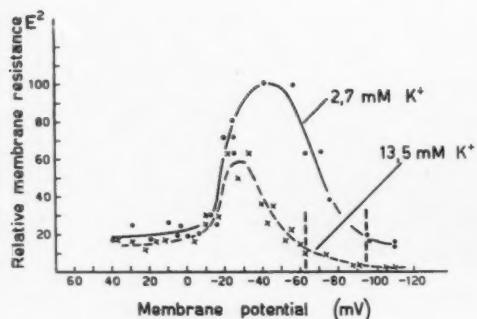


Figure 9

Membrane resistance as recorded from a sheep Purkinje fiber in a sodium-free solution. Note high resistance (=low membrane conductance) in the region of the normal plateau. Effect of K-rich solution on membrane resistance. Courtesy of Dr. E. Carmeliet, Louvain (unpublished).

might indeed be responsible for repolarization if it is attributed to a rise of  $G_K$ , thus resulting in a stronger outward current of K ions.

The finding of a strong outward current of tracer potassium ( $^{42}\text{K}$ ) during the phase of membrane repolarization<sup>18</sup> would seem to be in line with the electrical data.

Finally: do we know of any possible reason for which  $G_K$  might increase as a function of time when the membrane is held at a con-

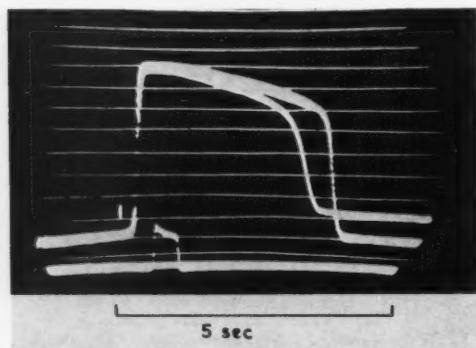


Figure 10

Shortening of the action potential of a turtle ventricle owing to a rapid rise of the K concentration in the perfusate. Potassium-rich solution was made to flow into the coronary artery for half a second, as indicated by the lowest trace. With a delay of  $1\frac{1}{2}$  seconds this initiated an early but incomplete repolarization. Voltage calibration lines from 10 to 10 mV. (From Weidmann.<sup>13</sup>)

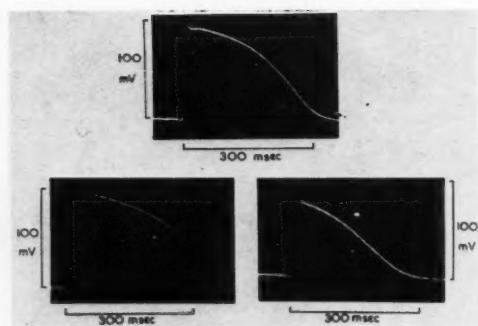


Figure 11

Effect of chloride substitution on the duration of the action potential. Sheep right ventricle. Upper record. Normal Tyrode solution. Lower left. Chloride ions substituted by acetylglycinate. Lower right. Chloride ions substituted by nitrate. Courtesy of Dr. E. Carmeliet, Louvain (unpublished).

stant potential in the region of the plateau? In this connection let me recall that a rapid rise of the extracellular K concentration induces a "premature" repolarization (fig. 10). One of the suggested mechanisms for this observation was recently tested by Carmeliet.<sup>19</sup> He was able to demonstrate that an increase in the extracellular K concentration causes a drop in membrane resistance that may be interpreted as a rise of  $G_K$  (fig. 9). The effect is most pronounced near -40 mV, i.e., in the region of the plateau of the action potential of a Purkinje fiber.

Furthermore, Carmeliet<sup>19</sup> measured the in- and outflux of radio-K using extracellular solutions of different K concentrations. He could indeed establish that the rate of influx as well as that of outflux became larger when the outside K concentration was increased, again suggesting a rise of  $G_K$ .

It seems not unlikely, therefore, that the plateau is brought to an end by the following mechanism: (a) outflux of K ions during the plateau; (b) accumulation of K ions in a narrow space around the fibers; (c) increase of  $G_K$  as a consequence of the rising extracellular K concentration; (d) increase in the rate of K outflux. This is a regenerative process and might well be responsible for terminating the cardiac action potential.

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## Physiology of Atrioventricular Transmission

By BRIAN F. HOFFMAN, M.D.

This paper describes records of the transmembrane action potential of fibers from different parts of the specialized conducting system and electrograms recorded directly from these fibers *in situ*. On the basis of these records it is possible to describe certain physiologic mechanisms for conduction delay, block, and supernormal conduction. In general, impaired conduction is associated with a reduced level of membrane potential. This may be caused by incomplete repolarization or partial depolarization. In the normal conducting system, local differences in action-potential duration and local pacemaker activity most frequently are the cause of a low membrane potential. In disease states, on the other hand, many other factors may be operative. At the atrial margin of the atrioventricular (A-V) node, local anatomic and electrophysiologic properties of the fibers normally cause a very low conduction velocity. The safety factors for conduction here appear to be quite low, and delayed transmission or block often does not result from refractoriness or partial depolarization of nodal fibers. Supernormal conduction, at least in Purkinje fibers, seems to result from the high level of membrane potential reached at the end of repolarization. Whether other factors are responsible for supernormality within the A-V node remains to be seen.

DURING the normal cardiac cycle, electrical activity originates in some part of the sinoatrial node and spreads to the atrium and thence to the atrioventricular node. After some delay, activity then spreads through the bundle of His, the bundle branches, and the peripheral Purkinje fibers and finally reaches the musculature of the ventricles. In the normal heart, activity that is initiated in some part of the ventricles can spread in the reverse direction along this same path. However, under a wide variety of abnormal conditions either sequence of events may not take place. Atrial activity may be delayed excessively at the atrioventricular node or may fail to excite the bundle of His. Activity that does traverse the atrioventricular node may be delayed or blocked in its passage through the specialized conducting system, and this delay or block may be localized to one or another anatomic subdivision. Also, unidirectional conduction delay or block may be observed. Finally, most and perhaps all parts of the specialized conduction system

may at times develop intrinsic rhythmicity and such ectopic pacemakers may compete with varying degrees of success with the normal sinus pacemaker.

A large number of comprehensive studies of normal and abnormal atrioventricular transmission has been carried out by careful analysis of the electrocardiogram. These studies have provided a fairly detailed picture of the physiology of the various parts of the atrioventricular (A-V) conducting system; however, the evidence in most instances has been somewhat indirect. This is so because electrical activity of the A-V node, the bundle of His, and the Purkinje fibers is not recorded directly in the conventional electrocardiogram. However, 2 methods can be used to demonstrate this electrical activity: through an intracellular microelectrode one can record the change in transmembrane potential associated with activity of a fiber from any part of the heart, and, by means of small electrodes placed directly over various parts of the specialized conducting system, one can record the local electrogram of the underlying structures.

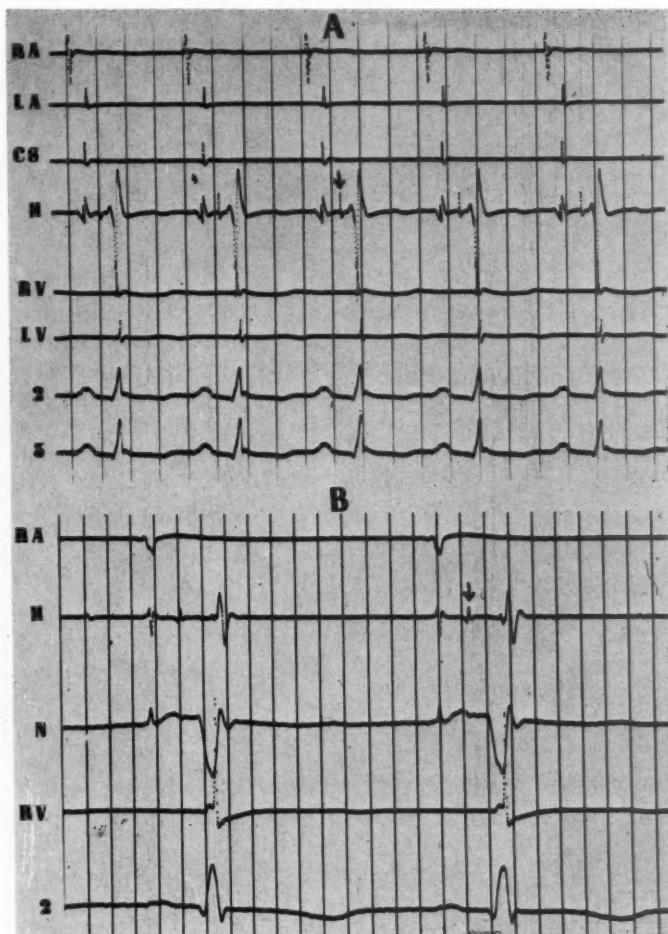
We have employed these 2 techniques to study certain aspects of the electrophysiology of fibers in the A-V node, the bundle of His, and the Purkinje system and to obtain some-

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Figure 1

A. Bipolar electrograms recorded through chronically implanted electrodes from dog heart and standard electrocardiograms. RA, right atrium; LA, left atrium; CS, coronary sinus; H, bundle of His; RV, right ventricle; LV, left ventricle; 2 and 3, leads II and III. Arrow indicates electrogram deflection caused by activity in the bundle of His. B. Records similar to those in A obtained from a different animal. RA, bipolar electrogram from the right atrium; H, bipolar electrogram from the bundle of His; N, unipolar electrogram from the region of the atrio-ventricular node; RV, bipolar electrogram from the right ventricle; 2, lead II electrocardiogram. For all bipolar electrograms low frequency components (i.e., under 40 c.p.s.) and components above 200 c.p.s. are strongly attenuated. The unipolar electrogram from the node and the electrocardiograms are recorded with the frequency response usual for electrocardiography. Time lines at intervals of 40 msec.



what more direct information on the physiologic mechanisms responsible for certain disturbances of A-V transmission. Experiments using microelectrodes were carried out on isolated preparations of rabbit or canine hearts. The methods have been described elsewhere in detail.<sup>1, 2</sup> Electrograms were recorded directly from various parts of the specialized conducting system of canine hearts *in situ* using small electrodes that had been attached to the endocardium during total cardiopulmonary bypass.<sup>3</sup> The experiments were acute in some instances; in others, records were obtained from healthy animals in which electrodes had been implanted previously.<sup>4</sup>

#### Activation of the Specialized Conducting System The Sequence of Activation

The exact sequence of activation of the specialized conducting system in canine hearts has been determined by several investigators from bipolar electrograms recorded by means of electrodes closely placed over the bundle of His, the right and left bundle branches, and the peripheral Purkinje fibers and from unipolar electrograms recorded by way of electrodes in close proximity to the A-V node.<sup>5-11</sup> In figures 1 and 2 are several such tracings recorded simultaneously with a standard limb-lead electrocardiogram. A fairly accurate estimate of the instant during the

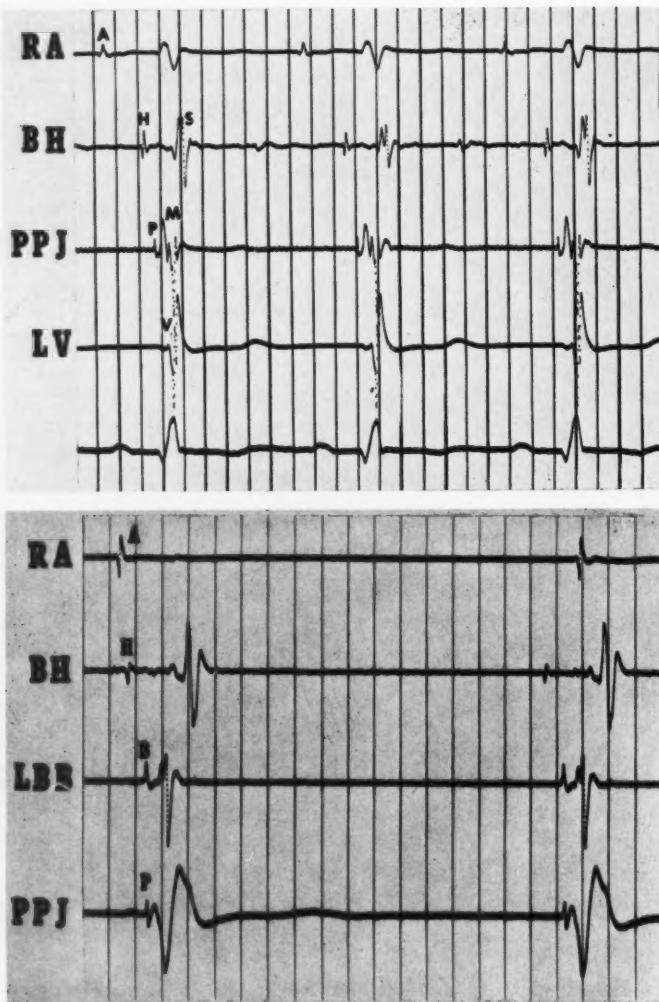


Figure 2

Top. Bipolar electrograms recorded through chronically implanted electrodes from right atrium (RA), bundle of His (BH), right Purkinje-fiber-papillary-muscle junction (PPJ), left ventricle (LV) and a standard lead II electrocardiogram. Deflections are labeled as follows: A, atrium; H, bundle of His; S, septal muscle; P, peripheral Purkinje fiber; M, papillary muscle. Low frequency components are filtered from the second, third, and fourth traces. Time lines at intervals of 40 msec.

Bottom. Bipolar electrograms recorded during an acute experiment through electrodes located on the right atrium, over the bundle of His, the left bundle branch (LBB) and the peripheral Purkinje fiber-papillary muscle junction. Deflections are labeled as in A. Time lines at intervals of 40 msec. Low frequency components are filtered from all traces.

cardiac cycle when atrial activity reaches the A-V node can be obtained by noting the time at which atrial depolarization is recorded through leads located either over the bundle of His or below the ostium of the coronary sinus (fig. 1A). From such records it is apparent that the A-V node is excited early during the P wave of the electrocardiogram. The time required for activity to traverse the node can best be determined by recording the onset of propagated activity in the upper end of the bundle of His. This approach is necessary because the electrical activity of the A-V

nodal fibers is not easily demonstrated in surface electrograms.<sup>6, 11</sup> Unipolar records from the node show a slow, predominantly positive deflection of low voltage (fig. 1B); however, it is likely that in many instances this deflection derives, in part, from currents associated with repolarization of nearby atrial muscle.

Electrodes located over the bundle of His, on the other hand, clearly signal the arrival of propagated depolarization in this structure. The electrogram deflection resulting from activity in the upper part of the common bundle,

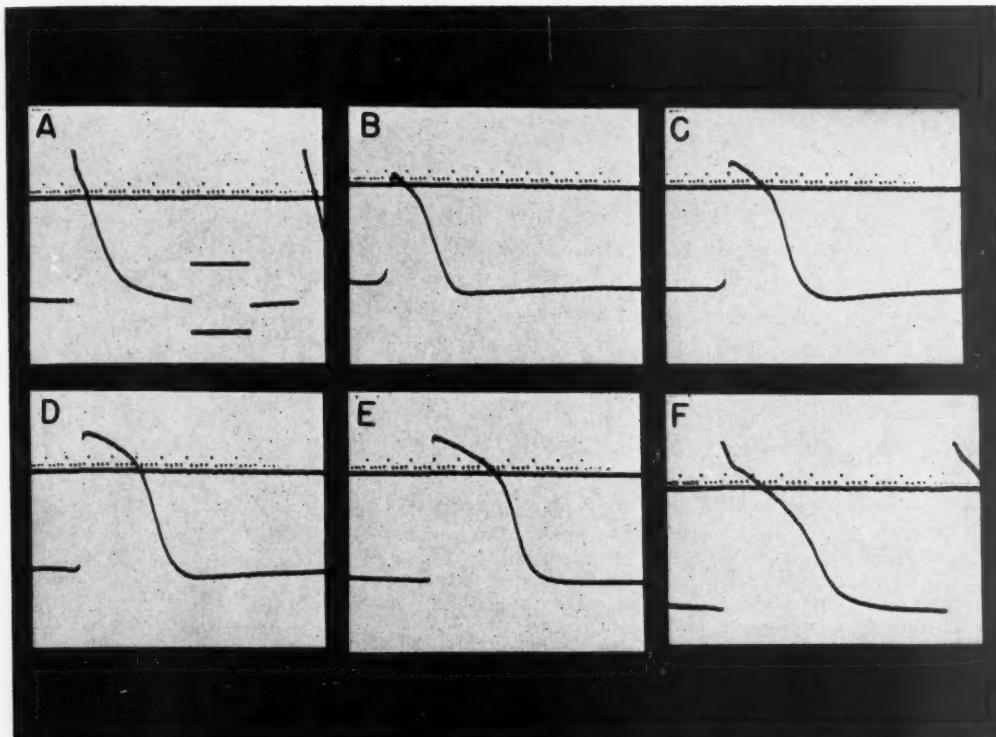


Figure 3

Transmembrane action potentials recorded from single fibers of atrium (A), upper node (B), mid and lower node (C, D, E), and upper of His bundle (F). Upper trace represents a line of zero potential and shows time calibration (dots) in intervals of 10 and 50 msec. Voltage calibration in A, from above down, shows in mv: -50 and -100. Overshoot in (A) is larger than that commonly recorded. (From Hoffman et al.<sup>12</sup>)

usually designated by the letter "H," appears during the first half of the P-R interval. Identification of the complexes resulting from activity in different parts of the specialized conducting system often is facilitated if low frequency components of the tracing are filtered. This device allows the use of high amplification to increase the relative magnitude of the rapid complexes that result from activity in the bundle of His or the Purkinje fibers. It has been employed for the records shown in many of the illustrations; in each instance the filter settings of the preamplifier are noted in the legend. The time required for A-V nodal transmission in dogs, determined from records like those in figure 1, ranges

from 40 to 50 msec. These findings have been the same both in acute experiments and in animals with chronically implanted electrodes.<sup>4, 5</sup> The electrogram recorded from the bundle of His varies somewhat in configuration and timing, depending on the location of the electrodes;<sup>6</sup> however, during normal A-V transmission, it is apparent that excitation of the various fibers in the common bundle is quite synchronous.

Records obtained through electrodes located over the right or left bundle branches, the free-running Purkinje fibers in the false tendons of the left ventricle, and the peripheral Purkinje fibers at their junction with the papillary muscles of the left or right ventricle can

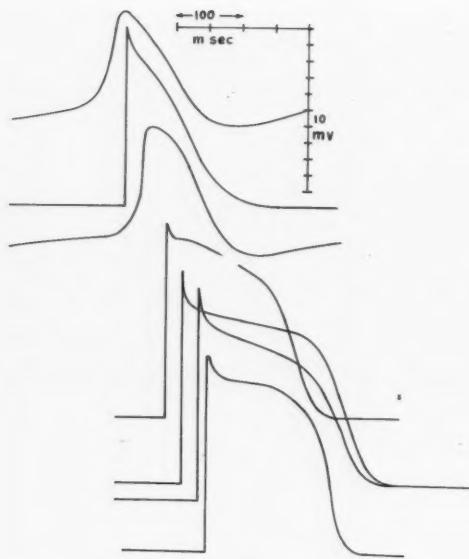


Figure 4

Drawings of transmembrane action potentials recorded from the following sites from above down: sinoatrial node, atrium, atrioventricular node (atrial margin), bundle of His, left bundle branch, Purkinje fiber in a false tendon, terminal Purkinje fiber, and ventricular muscle fiber. Note the sequence of activation at the various sites as well as the differences in the amplitude, configuration, and duration of the action potentials. (From Hoffman and Cranefield.<sup>2</sup>)

be employed to time the onset of activity in these structures (fig. 2). At each location the rapid deflection resulting from activity in the Purkinje fibers can be identified; since the recording electrodes are located progressively closer to the ventricular terminals of the specialized conducting system, the interval between this rapid deflection and the slower activity caused by depolarization of ventricular muscle decreases. The earliest activity recorded from the right or left bundle branches appears shortly after the midpoint of the P-R interval; that obtained from electrodes located at the Purkinje-fiber-papillary-muscle junction often is synchronous with the beginning of the initial deflection of the standard electrocardiogram. Electrodes located at appropriate sites on the endocardial surfaces of the ventricles record the local ar-

rival of depolarization in the subendocardial Purkinje-fiber network as small rapid deflections that precede local ventricular activity by an interval of a few milliseconds.

#### Conduction Velocity

When the time in the cardiac cycle of electrograms recorded from each part of the specialized conducting system is considered in relation to the distance between recording sites, it is apparent that conduction velocity varies considerably during the spread of the impulse from the atria to the ventricles. Extremely slow propagation through the A-V node has been postulated for many years; recent studies of perfused dog hearts<sup>11</sup> and isolated preparations of dog and rabbit heart<sup>2, 12</sup> have shown that, at the atrial margin of the node, the conduction velocity apparently falls to the extremely low value of 0.05 M./sec. or less. During normal A-V transmission the major delay in propagation is localized to the atrial margin of this structure. Within the node, conduction velocity increases progressively and in the bundle of His attains a value of 1.0 to 1.5 M./sec.<sup>13</sup> It is likely that the rapidity of spread of the impulse increases progressively toward the periphery of the common bundle. Measurements of conduction velocity in the free-running Purkinje fibers in the right and left ventricles of canine hearts, obtained during cardiopulmonary bypass, give values for conduction velocity ranging from 3 to 4 M./sec. In the fine terminal ramifications of the Purkinje system, conduction velocity decreases and, in ventricular muscle, velocity is approximately 1 M./sec. During retrograde activation, which results from premature ventricular activity late in the cycle or from an idioventricular pacemaker firing at a low frequency, there is no demonstrable change from normal in the conduction velocity in the Purkinje fibers and in the bundle of His. Retrograde transmission from the A-V node to the atrium is slower than that recorded during normal activity,<sup>11</sup> and again the delay appears to be localized to the atrial margin of the node. During such retrograde activation of the specialized con-

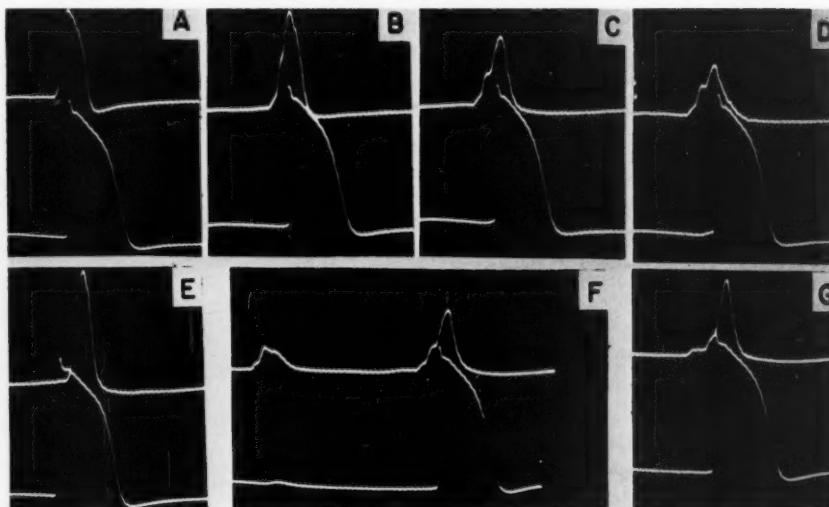


Figure 5

Transmembrane action potentials recorded from single fibers of the atrial margin of the atrioventricular node (upper trace) and bundle of His (lower trace) in an isolated preparation of rabbit heart. A. Control; B-G, effects of acetylcholine added to perfusion fluid. Note progressive increase in slurring and notching of upstroke of nodal potential (B-D), normal nodal action potential caused by retrograde activation (E) and fragmentation of nodal response in F and G. (From Cranefield, Hoffman, and Paes de Carvalho.<sup>19</sup>)

duction system, local electrograms reveal 2 changes. The complex recorded from the bundle of His may show some slight asynchrony of activation of various fibers or fiber groups. Also, the complexes recorded from the region of the A-V node show a deflection that is not observed during normal transmission<sup>6</sup> and that may represent slow or delayed activation of atrial fibers at the atrionodal junction.

#### Transmembrane Action Potentials of Different Fibers

Probably the most useful record of electrical activity of cardiac fibers is the record of transmembrane potential obtained through an intracellular microelectrode. Transmembrane action potentials have been recorded from single fibers in isolated preparations taken from all parts of the specialized conducting system.<sup>1, 2</sup> Unfortunately, it has not yet been possible to obtain such records from the heart *in situ*. Also, although the peripheral Purkinje system of the adult canine heart has been studied extensively, many of the

records from the bundle of His and A-V node have been obtained from the hearts of rabbits or puppies. This resort to small animals is an outcome of the large mass of these structures in the adult dog heart, which prevents adequate perfusion of the isolated tissues. Nevertheless, it is possible to present a reasonably complete picture of the electrical activity of the single fibers present in each of the major subdivisions of the conducting system.

#### The A-V Node

Transmembrane action potentials recorded from fibers located in different parts of the A-V node are compared to a record of an atrial transmembrane action potential in figure 3.\* Several differences are apparent. The resting potential of the nodal fibers is lower than that of the atrium, the rate of rise of

\*Figure 3 reproduced from Hoffman et al.: Circulation Research 7: 11, 1959.<sup>12</sup> By permission of the American Heart Association, Inc.

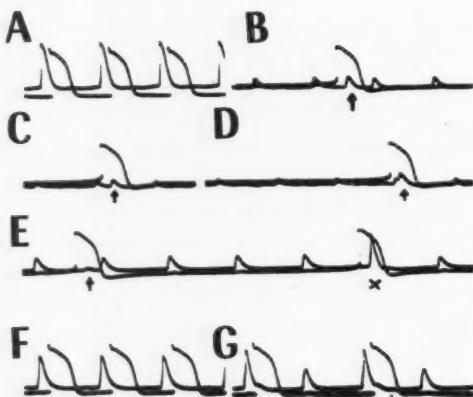


Figure 6

Transmembrane action potentials recorded from single fibers of the atrioventricular node (upper trace) and bundle of His (lower trace) of an isolated preparation of the rabbit heart. A. Control; B-D, after addition of acetylcholine to the perfusion fluid; E-G, during washout of acetylcholine. Note the small depolarization of the nodal fiber resulting from atrial activity and also from activity in the bundle of His (arrows). These subthreshold depolarizations summate (D) and elicit a nodal action potential if of sufficient amplitude (as at x in E).

the nodal action potential is much less, and the overshoot is reduced in amplitude. At the atrial margin of the node, records of transmembrane action potential often reveal one or more steps or notches on the upstroke.<sup>12, 14, 15</sup> The recorded electrical activity differs in different parts of the node;<sup>12</sup> the characteristics mentioned are most prominent at the atrial margin and become less pronounced in records obtained from fibers located closer to the bundle of His. The duration of the action potential is greater in the lower node than at the atrial margin of this structure and some slow diastolic depolarization is present in all records. Studies of conduction velocity within the node<sup>12, 16</sup> have shown that extreme slowing is present only in the fibers at the atrial margin, i.e., in those fibers whose action potentials show the lowest rate of rise and lowest amplitude. Both of these properties of the nodal action potential—reduced amplitude and low rate of rise—would decrease conduction

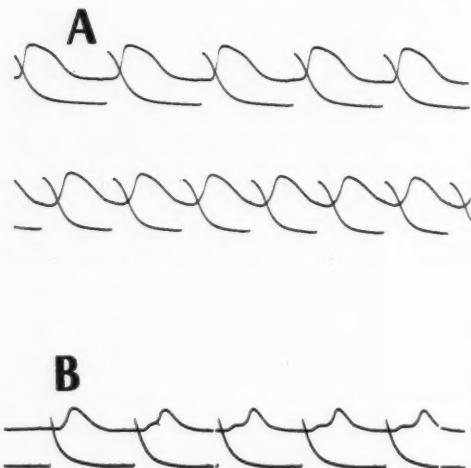


Figure 7

A. Transmembrane action potentials recorded from atrioventricular node (upper trace) and atrium (lower trace) of puppy heart showing changes in nodal action potential caused by high rate. B. Similar records recorded from a different preparation. In this experiment the nodal action potential is recorded somewhat closer to the lower node and the perfusion fluid contains a low concentration of acetylcholine. The first action potential is the last beat at a slow rate, the subsequent potentials are caused by a rapid driving rate. Note the distinct step on the upstroke of the nodal action potential.

velocity. It is not known whether the extremely slow propagation of activity in this part of the node results mainly from the characteristics of the action potential or in part also from the anatomic and passive electrical properties of the fibers. A-V nodal fibers in canine hearts are 6 microns or less in diameter<sup>17</sup> and, in some areas, have branches or extensions that are still smaller.<sup>18</sup> In small fibers, other factors being the same, conduction velocity is reduced because the resistance to flow of current along the axis of the fiber is high. Accurate measurements of the membrane resistance and capacity and the threshold potential of fibers at the atrial margin of the node have not been made, and thus it is impossible to evaluate the extent to which the unusual action potential and conduction velocity depend upon these properties. How-

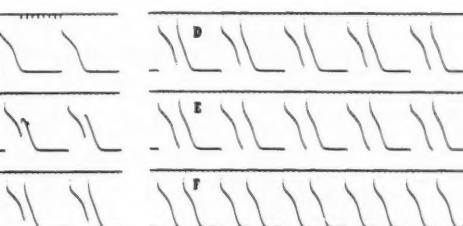
Figure 8

Transmembrane action potentials recorded from an isolated canine Purkinje fiber, showing the change in configuration and amplitude of responses to depolarizing pulses applied at various times during and after repolarization. Upward voltage step in A represents +100 mv. Time marks on upper trace at intervals of 100 msec.

ever, even if one assumes that they are much the same as in other cardiac fibers, the safety factor for transmission in this part of the node undoubtedly is reduced.

#### The Bundle of His and the Purkinje Fibers

The action potential recorded from a single fiber in the bundle of His of the rabbit heart contrasts quite markedly with the nodal action potential (figs. 3 and 4\*). The resting potential is higher, the upstroke of the action potential is rapid, and the amplitude is greater. The duration of the action potential is increased, and slow diastolic depolarization is minimal under ordinary conditions. The rapid depolarization and good amplitude of the action potential, in combination with the greater diameter of the fibers, undoubtedly are responsible for the increase in conduction velocity as activity spreads into this structure. Action potentials recorded from Purkinje fibers in the bundle branches and peripheral branches of the conducting system show other changes. There is a further increase in rate of rise and a small increase in the amplitude of the action potential in the bundle branches and false tendons, and then a progressive decrease in the terminal Purkinje fiber network. The duration of the action potential also increases with increasing distance from the common bundle and then decreases as the junction of the Purkinje fiber with ventricular muscle is approached (fig. 4). Diastolic depolarization is progressively less marked in records obtained from more peripheral fibers. Comparative data on fiber diameter and frequen-



cy of branching at different locations in the Purkinje system are not available. It is likely, however, that both factors (large diameter and infrequent branching) contribute to the high conduction velocity in the false tendons.

At the extreme periphery of the Purkinje system, records of the transmembrane action potential show all gradations between a typical Purkinje-fiber action potential and action potentials of ventricular muscle fibers. It is reasonable to assume that this finding results from a progressive change in the membrane properties that parallels the gradual change in histologic structure.<sup>18</sup> Action potentials recorded from ventricular muscle differ from those of the Purkinje system primarily in that the rate of rise is less, the amplitude and duration are somewhat less, and there is a steady level of membrane potential during diastole. Ventricular fibers are smaller in diameter, branch more frequently, and conduct at a lower velocity than do Purkinje fibers.

#### Physiologic Basis for Disturbances in Conduction

An attempt to present a detailed description of the physiologic mechanisms responsible for any of the disturbances in A-V transmission observed in the clinic certainly is premature. On the other hand, it is possible to describe the changes in electrical activity that have been observed in association with experimental conduction disturbances and to indicate the extent to which these changes might cause certain electrocardiographic alterations.

#### Disturbances of A-V Nodal Transmission

Records from isolated preparations of A-V nodal tissue of rabbit and dog heart have shown that most changes in conduction through the

\*Figure 4 reproduced from Hoffman and Cranefield: *Electrophysiology of the Heart*.<sup>2</sup> By permission of the McGraw-Hill Book Company.

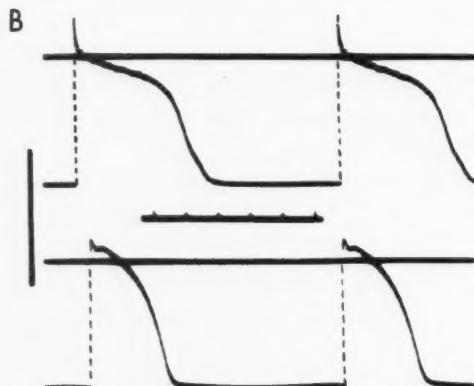


Figure 9

Transmembrane action potentials recorded from single fibers of Purkinje system (upper record) and ventricle (lower record) in an isolated preparation of canine heart. Time calibration between records shows intervals of 100 msec., vertical bar at left shows voltage calibration of 100 mv. Up-strokes of action potentials have been retouched with dashed lines.

node and most instances of block are associated with altered electrical activity of fibers at the atrionodal junction.<sup>2, 14</sup> A typical example is the delay and block of nodal transmission caused by acetylcholine. Action potentials recorded from fibers in the lower node and bundle of His show only those changes that are produced by the acetylcholine-induced change in frequency.<sup>19</sup> Transmembrane action potentials recorded from nodal fibers at the atrial margin, on the other hand, are profoundly altered. Under the influence of acetylcholine, the action-potential upstroke becomes slower and more notched, and the action potential decreases in amplitude (figs. 5\* and 6). Often it is replaced by 1 or more small depolarizations that vary in size and to a greater or lesser degree undergo temporal summation. Delay and failure of transmission undoubtedly result from these changes; however, the exact cause of the changes noted is less certain.

During complete failure of A-V transmis-

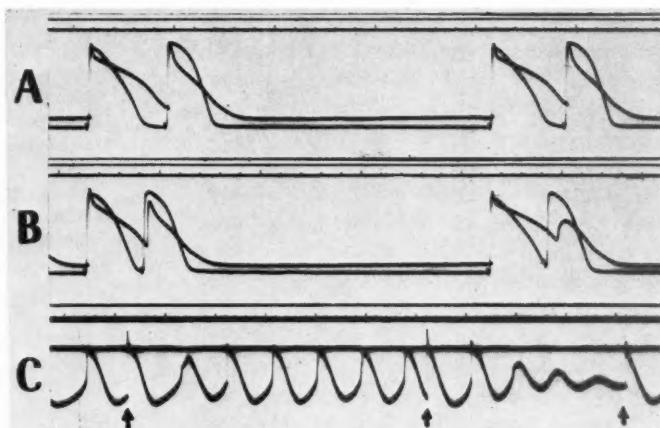
\*Figure 5 reproduced from Cranefield et al.: Circulation Research 7: 19, 1959.<sup>19</sup> By permission of the American Heart Association, Inc.

sion owing to acetylcholine, activity originating in the bundle of His and propagating back to the same nodal fibers elicits an action potential of good amplitude (figs. 5 and 6). Moreover, the upstroke of this action potential often is free from slurring or notching even when these changes were prominent during normal A-V transmission. Although direct measurements of threshold are lacking, some estimate of the effect of acetylcholine on excitability of the nodal fibers can be obtained from an inspection of records similar to those in figure 6. The small depolarizations recorded during partial block show temporal summation; comparison of the level of depolarization caused by such summation, which is just sufficient to cause propagation through the node, with the level of membrane potential at which the action potential shows an abrupt transition from slow to rapid depolarization suggests that the threshold potential is not much changed by acetylcholine. It does not increase the resting potential of fibers in the A-V node as it does in the sinoatrial node; this observation at least does not give any positive support to the possibility that acetylcholine causes block by decreasing membrane resistance of nodal fibers. The block caused by acetylcholine thus appears to result from the failure of the fibers at the atrial margin of the node to develop an action potential, and this failure is associated in some way with asynchronous excitation of these fibers. Both the failure of excitation and the asynchronous excitation may be due to the action of acetylcholine on the atrial fibers at the atrionodal junction. Action potentials recorded from them are greatly decreased in amplitude during acetylcholine-induced block. This may be caused by the effect of acetylcholine on potassium permeability which is known to occur in atrial muscle: a greatly enhanced  $K^+$  efflux may partly cancel the depolarization caused by an inward  $Na^+$  current.<sup>20</sup>

Disturbances of A-V transmission caused by premature atrial beats or a rapid atrial rate are associated with somewhat different changes in nodal action potentials. At the

Figure 10

**A and B.** Transmembrane action potentials recorded from an isolated Purkinje-fiber-papillary-muscle preparation of canine heart. Extrasystoles in the papillary muscle (lower trace) excite the Purkinje fiber at various levels of membrane potential during repolarization and elicit either premature action potentials or local responses (last action potentials in B). **C.** Transmembrane action potentials recorded from an isolated preparation of canine Purkinje fibers. Marked pacemaker activity has been induced by an excessive concentration of digitalis. Extrasystoles (arrows) caused by test pulses contrast markedly in terms of rate of rise and amplitude of the action potential with the action potentials of intrinsic origin.



atrial margin of the node, the amplitude of the transmembrane action potential is reduced and the rate of depolarization is slowed (fig. 7A). When these changes are extreme, records from fibers in the lower node may show a slow, steplike depolarization of considerable duration preceding the local action potential (fig. 7B). During failure of transmission, only the graded steplike depolarization is recorded. Block caused by agents such as digitalis or quinidine is associated with similar changes in nodal action potentials.<sup>15</sup> During block of retrograde impulses that reach the node, the failure of conduction most often is localized to the atrionodal junction. Also, action potentials recorded at different sites within the node are different during normal and retrograde transmission;<sup>16</sup> the change is in the initial segment of the upstroke and probably is related to the anatomic arrangement of the various fibers.

#### Delay and Block Within the Bundle of His and Bundle Branches

Experimentally produced conduction disturbances within the bundle of His and bundle branches most often result from 2 factors: local differences in action-potential duration and/or the presence of slow diastolic depolarization associated with latent pacemaker ac-

tivity. In both cases the failure of normal conduction results directly from the low membrane potential.<sup>21</sup> If the transmembrane potential is reduced, because of either incomplete repolarization or local pacemaker activity, the rate of rise and amplitude of the action potential are decreased (fig. 8). The altered action potential may propagate at a reduced velocity or may constitute a purely local response. If the reduced conduction velocity permits the adjacent membrane to repolarize completely, the slowing of conduction may be localized to a small segment of the conducting system. If, on the other hand, slowing of conduction is caused by diastolic depolarization, adjacent areas of membrane will have reached still lower levels of membrane potential and decremental conduction and block may result.

#### Block at the Junction of Purkinje Fibers with Ventricular Muscle

During A-V transmission, block at the junction of Purkinje fibers with ventricular muscle fibers is unlikely for several reasons. The transition from the larger Purkinje fibers to the smaller muscle fibers is gradual, and thus there is not an abrupt increase in the area of excitable membrane. Also, the duration of the ventricular action potential is consider-

ably less than that of the Purkinje fibers in adult mammalian hearts (fig. 9). Hence the likelihood of a premature impulse reaching the ventricle before it is fully repolarized is reduced. However, premature depolarization of ventricular muscle may be delayed or blocked at the junction with the Purkinje system (fig. 10, A and B) and the impulse may propagate at reduced velocity for a considerable distance. Although a conclusive experimental demonstration is lacking, it is likely that excitation may enter some branches of the Purkinje system and fail to enter others. Whether this would cause local re-excitation of the ventricle has not yet been determined.

#### Supernormality

The term "supernormality" is used with 2 meanings in descriptions of cardiac excitability: it may refer to a reduced stimulus requirement or it may refer to conduction that is either faster than expected or takes place under conditions that might be expected to cause block. The cause of both forms of supernormality is clear from studies of transmembrane action potentials.<sup>21, 22</sup> Enhanced excitability is found during the terminal phase of repolarization; however, the action potential elicited at this time is reduced in amplitude and propagates slowly. A supernormal phase of conduction is observed in fibers which are partially depolarized or in which there is appreciable diastolic depolarization. In such fibers, membrane potential reaches its highest value just at the end of repolarization. An action potential elicited at this moment will show a higher rate of rise and greater amplitude than responses that occur later during the cardiac cycle (fig. 10B). Also, the larger action potentials will propagate more rapidly and will have a greater safety factor.

#### Acknowledgment

Many of the studies on which this article is based were carried out in collaboration with either Paul F. Cranefield or Jackson H. Stuckey; it is a pleasure to acknowledge the major contributions they have made to these experiments and to many of the concepts that are expressed in this paper.

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## Calcium Movements in Muscle

By C. PAUL BIANCHI, PH.D.

The movements of calcium in muscle have been followed during contraction and contracture to test the hypothesis that the release of calcium from the surface of the muscle membrane during stimulation initiates the contractile mechanism. Nitrate ion increases the calcium influx during a single twitch and during potassium contracture, and also increases the tension developed. The increased entry of calcium during a potassium contracture is transient and not sustained as is the contracture. Caffeine, which brings about a contracture without depolarization of the membrane and despite the absence of calcium from the medium, causes calcium to be released from the muscle.

THE PRESENT DISCUSSION of calcium movements in muscle will deal with frog sartorius muscle. In the next paper, Dr. Winegrad will consider recent findings on heart muscle.

Calcium has long been proposed as the link between membrane depolarization and contraction.<sup>1-4</sup> Of all the physiologic ions that have been injected in small quantities, calcium alone causes contraction.<sup>5, 6</sup> Sandow, in 1952,<sup>2</sup> made a detailed correlation of the kinetics of the sequence of excitatory and mechanical events (fig. 1\*). At 13 C. the rise time of the spike potential is 0.6 msec.; the time from the peak of the spike potential to the onset of latency relaxation is 1.2 msec.; and it is during these time intervals that the muscle is mechanically quiescent. The time interval until the earliest sign of development of tension, the inflection point of latency relaxation, is 3.5 msec.; and the total time until the onset of tension above the initial tension is 5.4 msec. At 25 C. the corresponding time intervals are approximately 0.2 msec., and 2.5 msec. respectively. It is during the mechanically quiescent period that 2 processes are occurring: (1) membrane depolarization, and (2) an intervening process between the peak of the action potential and the beginning of latency relaxation termed the "spike activation" link.<sup>2</sup> The size of the spike potential appears to be unrelated to twitch tension for anions that potentiate the twitch,

e.g., Br, NO<sub>3</sub>, I, SCN, CH<sub>3</sub>SO<sub>4</sub> have been shown to have little effect on the spike.<sup>3, 7, 8</sup> Hodgkin and Horowicz<sup>9</sup> have found agreement between the threshold of depolarization necessary for mechanical activity during K-contracture and the degree of depolarization necessary to initiate the spike potential, suggesting that it is the lowering of the membrane potential to a critical level, rather than the height of the spike potential, that is concerned in excitation-contraction coupling.

Under physiologic conditions, the conducted action potential of frog sartorius muscle initiates a process whereby contraction occurs. Membrane depolarization itself is not the most intimate link in the process, for contraction can be brought about without depolarization of the muscle membrane. Thus, caffeine can cause a contracture without any change occurring in the resting membrane potential.<sup>10</sup> Potassium-induced contracture can fail to occur, even though the muscle membrane is depolarized, if external calcium is removed.<sup>11</sup> Csapo has shown that treatment of the turtle retractor penis muscle with NaI can bring about greater tension development with smaller membrane depolarization.<sup>12</sup> Hodgkin and Horowicz have demonstrated that replacement of Cl with NO<sub>3</sub> can lower the threshold of membrane depolarization necessary to bring about a contraction.<sup>13</sup> All of these findings point to another intervening process between membrane depolarization and the initiation of mechanical activity.

Studies on calcium movements in muscle

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\*Figure 1 from Sandow: Yale J. Biol. & Med. 25: 176, 1952.<sup>2</sup> By permission of the journal.

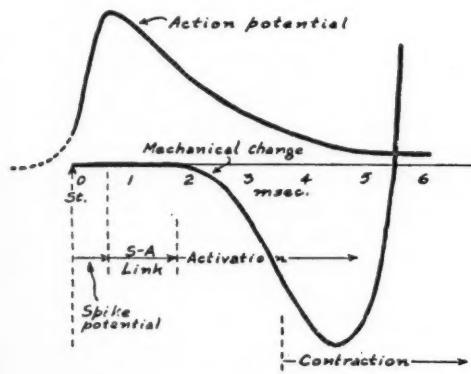


Figure 1

Temporal correlation of excitation and mechanical events during latent period of frog sartorius muscle at 13°C according to Sandow. (From Sandow.<sup>2</sup>)

conducted by Shanes and Bianchi provide evidence that an increase in calcium influx during depolarization by an action potential, or by an increase in extracellular K, is part of the intermediate process between events in the muscle membrane and mechanical activity of the contractile proteins. Table 1 shows that calcium influx in unstimulated muscle is 0.094 micromicromoles/cm.<sup>2</sup>sec. Stimulation of the muscle causes additional calcium to enter the muscle, which amounts to 0.2 micromicromoles/cm.<sup>2</sup> twitch. If one assumes that calcium enters the muscle fibers at a high rate immediately upon depolarization and continues to enter during the period of mechanical quiescence (1.0 msec. at 25°C.), then the resting influx rate of calcium would have increased from 0.1 micromicromoles/cm.<sup>2</sup> sec. to 200 micromicromole/cm.<sup>2</sup> sec., an increase of approximately 2,000. If the calcium were considered to enter during the period from membrane depolarization to the appearance of initial tension development (2.5 msec.), then the increase would still be 800-fold.

The amount of calcium entering per twitch and the twitch height is increased 60 per cent by replacing the chloride of Ringer's solution with nitrate. Under these conditions, nitrate has no effect on the unstimulated influx of calcium, showing that nitrate affects only the

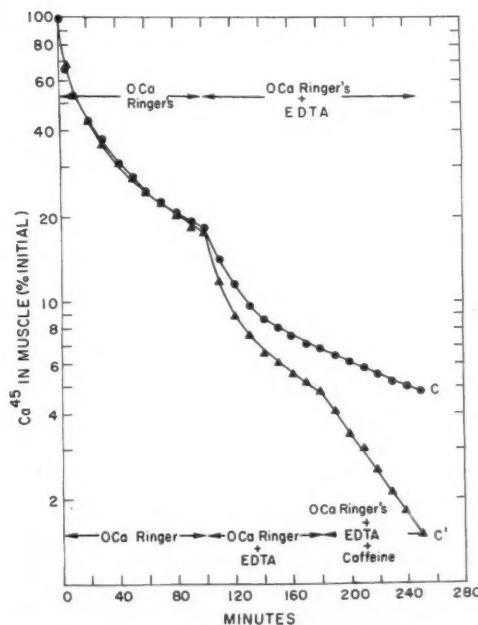


Figure 2

The effect of edathamil (EDTA) and caffeine on the washout of  $\text{Ca}^{45}$  from a muscle previously soaked for 5 hours in  $\text{Ca}^{45}$  Ringer's solution. At 100 minutes 0.004 M EDTA is added to the medium, bathing both C and C'. An immediate increase in  $\text{Ca}^{45}$  release occurs, which tapers off after 140 minutes. At 180 minutes, 0.005 M caffeine is added to muscle C', causing a sustained increase in the release of  $\text{Ca}^{45}$  from the muscle.

calcium entering during a twitch. Nitrate has been shown to prolong the active state,<sup>14, 15, 16</sup> which would account for the increased twitch height that is observed in nitrate Ringer's solution. A transitory increase in the ionized calcium level of the muscle fibers, brought about by the increased amount of calcium entering per twitch, could account for the prolongation of the active state. In keeping with this hypothesis, superficial muscle-fiber sites have been shown to bind about 0.1 micromole/Gm. of calcium in nitrate Ringer's solution.<sup>17</sup> The increased binding of calcium and the increased influx during stimulation are in agreement with the suggestion of Shanes<sup>4</sup> that the enhancement of the twitch height when other halogens or nitrate replaces chloride may be due to an improved binding of

Table 1  
Calcium Influx in Frog Sartorius Muscle

Conditions	Influx
Unstimulated	
Ringer's solution	0.094 $\mu\text{mole}/\text{cm.}^2 \text{ sec.}$
Ringer's nitrate solution	0.108 $\mu\text{mole}/\text{cm.}^2 \text{ sec.}$
Stimulated	
Ringer's solution	0.20 $\mu\text{mole}/\text{cm.}^2 \text{ twitch}$
Ringer's nitrate solution	0.32 $\mu\text{mole}/\text{cm.}^2 \text{ twitch}$
Ringer's solution + 20 mM KCl	0.124 $\mu\text{mole}/\text{cm.}^2 \text{ sec.}$
Ringer's solution + 80 mM KCl (after relaxation)	0.056 $\mu\text{mole}/\text{cm.}^2 \text{ sec.}$
Ringer's solution + 80 mM KCl (initial depolarization)	38 $\mu\text{mole}/\text{cm.}^2$
Ringer's nitrate solution + 80 mM KNO <sub>3</sub>	60 $\mu\text{mole}/\text{cm.}^2$

calcium to the membrane, which in turn contributes to enhanced entry of calcium during stimulation.

Depolarization of the sartorius muscle by 20 mM K, a level just below the threshold for contracture, leads to a sustained increase in calcium influx (table 1). Calcium influx measured in the presence of 80 mM K, after relaxation from the induced contracture, is smaller than calcium influx in 20 mM K, and almost equal to influx in unstimulated muscle. The entry during initial depolarization in 80 mM K amounts to 38 micromicromole/cm.<sup>2</sup>.<sup>18</sup> The presence of nitrate increases the amount to 60 micromicromole/cm.<sup>2</sup>, which is in keeping with the potentiated contracture that is observed.<sup>19</sup> The large transient increase in calcium influx during initial depolarization is consistent with the rapid increase in tension during the first second of KCl contracture observed by Hodgkin and Horowicz.<sup>9</sup> The relaxation of the phasic muscle during maintained KCl depolarization can be interpreted as a failure to sustain the high rate of calcium influx. Shanes<sup>20</sup> has shown that a high rate of calcium entry persists in the slow fibers of the frog rectus abdominis, along with the sustained potassium contracture. Thus, in fast fibers both the increased influx of calcium and the contracture are transient during K depolarization, whereas both are sustained in slow fibers during K depolarization.

Contracture brought about by caffeine has important differences from potassium-induced contracture. Potassium contracture is not sustained and is associated with a transitory increase in calcium influx during the initial

membrane depolarization. Removal of external calcium prevents potassium contracture.<sup>11</sup> In contrast, caffeine causes a sustained contracture in frog sartorius without membrane depolarization and in the complete absence of external calcium. The site of caffeine action is on the membrane. Axelsson and Thesleff<sup>10</sup> have shown that only caffeine applied externally to the membrane results in a contracture, while caffeine applied by injection to the muscle interior is without effect. It has also been shown that caffeine markedly increases calcium outflux and influx.<sup>18</sup> Figure 2 shows that even after prior treatment of frog sartorius muscle with edathamil (EDTA), which can remove some superficial, bound calcium as well as calcium in solution, caffeine causes a marked increase in calcium outflux, suggesting that caffeine can bring about the release of calcium from membrane sites and perhaps sarcoplasmic reticulum sites, which in turn results in contracture. The increased calcium outflux may therefore reflect the freeing of bound calcium, which would raise the intracellular calcium ion content and thus induce contracture without the necessity of external calcium or a membrane depolarization.

The release of calcium during stimulation has been observed by Woodward<sup>21</sup> and confirmed by Shanes and Bianchi.<sup>22</sup> Figure 3 clearly demonstrates the release of calcium during tetanic stimulation. Potassium contractures, both isotonic and isometric, increase calcium outflux (fig. 4). The increased outflux during tetanic stimulation is not sustained and the minimum calcium released per twitch is about the same as the amount taken

up per twitch: viz., 0.2 micromicromole/cm.<sup>2</sup><sup>22</sup>

Potassium contracture results in a rapid release of calcium, which is at about double the base line rate even after 10 minutes (fig. 4). The increased influx and outflux of calcium in frog sartorius observed with tetanic stimulation or potassium contracture may reflect the same basic process, such as freeing of calcium from the surface, supported by the rapid release of calcium during tetanic stimulation. Two other possible explanations for the increased influx and outflux are: (1) a spatial separation in which different sites of the membrane involve calcium influx and outflux, and (2) a temporal separation of the two fluxes.

From the foregoing, it is evident that calcium influx into the muscle fiber is related to mechanical activity in 2 ways. The enhanced twitch height and contracture in nitrate Ringer's solution is correlated with a larger influx of calcium, and there is a temporal relationship between the duration of increased calcium influx and of mechanical activity. Potassium contractures and a high rate of calcium influx are transitory in phasic muscles, while in slow fibers potassium contractures are sustained as is the high rate of calcium influx. The manner in which calcium brings about activation of the contractile mechanism is still unknown, although from experiments on model systems there appear to be 2 possible modes of action. One would be the inhibition of the relaxing factor system, thus allowing contraction to take place, with relaxation occurring as the ionized calcium is removed; the other would be by a direct action of calcium on actomyosin. Weber<sup>23</sup> has shown that calcium in a concentration of  $10^{-4}$  M, which would be equivalent to  $5 \times 10^{-5}$  M ionized calcium, gives a maximum activation of the highly purified actomyosin ATPase system and also maximum superprecipitation of actomyosin with 2 mM Mg ATP. In the absence of added calcium, no superprecipitation could be measured, and the ATPase activity was reduced to 20 per cent of the maximum activity. There is, however, a large discrepancy between the

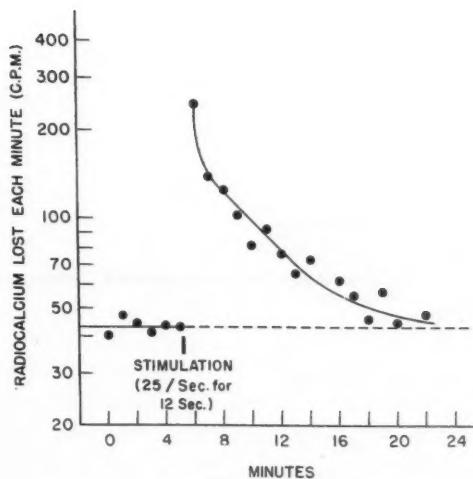


Figure 3

The release of  $\text{Ca}^{45}$  during a brief tetanic stimulation from frog sartorius muscle that has been previously soaked in  $\text{Ca}^{45}$  Ringer's solution. Sample collections of the medium bathing the muscle were made at minute intervals in order to obtain the true time course of calcium release.

amount of calcium needed for either inhibition of the relaxing factor system or activation of the actomyosin ATPase system. If the calcium entering per twitch (0.2 micromicromole/cm.<sup>2</sup>) were uniformly distributed in the muscle fiber water, then the final concentration of ionized calcium would be  $10^{-7}$  M, which is too small a concentration by a factor of 100 for both the relaxing factor system and the actomyosin ATPase. The discrepancy is still larger when the number of calcium ions that enter in relation to the actomyosin concentration of muscle is considered. If one estimates 100 mg. of actomyosin for every gram wet weight of muscle and a molecular weight of 500,000, then the actomyosin concentration of frog muscle would be approximately  $2 \times 10^{-4}$  M, as compared to  $10^{-7}$  M for calcium. Much more ionized calcium would be needed than can be accounted for by the calcium entering per twitch, suggesting perhaps that the initial entry of calcium from the membrane can bring about a further release of calcium from binding sites localized in the sarcoplasmic reticulum.

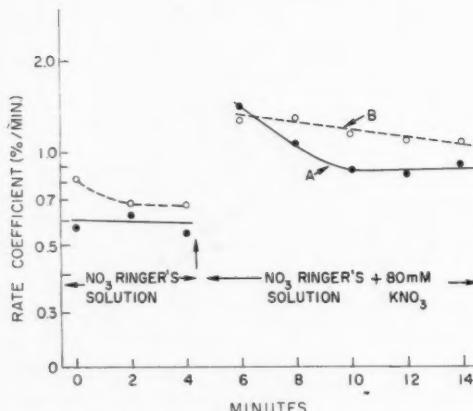


Figure 4

Comparison of the time course of calcium release during isotonic (A) and isometric (B) contracture. The calcium released is plotted as a rate coefficient, i.e., the percentage of the exchangeable calcium in the muscle being released during the 2-minute collection intervals. The contracture occurs when 80 mM  $\text{KNO}_3$  is added to the  $\text{NO}_3$  Ringer's solution.

Thus, calcium influx is markedly increased during a muscle twitch and potassium contracture in frog sartorius muscle. Nitrate ion, which potentiates both the twitch and potassium contracture, also increases the entry of calcium under these conditions. The caffeine-induced contracture may be accounted for by an increase in the ionized calcium level in the muscle fiber brought about by a direct action of caffeine on calcium-binding sites in the membrane and perhaps those located in the sarcoplasmic reticulum.

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# The Possible Role of Calcium in Excitation-Contraction Coupling of Heart Muscle

By SAUL WINEGRAD, M.D.

The transfer of  $\text{Ca}^{45}$  at rest and during contraction has been measured in isolated guinea-pig atria. During contraction, the rate of transfer increases considerably. The increment in the uptake of calcium by the cells of the atria during contraction is closely correlated with the strength of contraction. This relationship is maintained at different frequencies of stimulation and at different concentrations of external calcium.

THE IMPORTANCE of calcium in the contraction of heart muscle has been known since the experiments of Ringer.<sup>1</sup> Early work<sup>2,3</sup> focused primarily on the dependence of the strength of contraction on the concentration of calcium ions in the bathing solution. More recently an antagonism between sodium and calcium ions at the cell surface has been inferred from the observation that the effects of a decrease in extracellular sodium concentration and an increase in extracellular calcium concentration on twitch tension are similar.<sup>4</sup> The observation that withdrawal of calcium ions from the bathing solution caused rapid disappearance of mechanical but not of electrical activity of isolated heart muscle implicated the calcium ion as the excitation-contraction link.<sup>5</sup>

In a series of studies on frog ventricular strips, Niedergerke and Lüttgau<sup>6</sup> showed that changes in external sodium and calcium concentrations very rapidly altered the characteristics of potassium-induced contracture and that these changes in contracture tension could be produced even after the initial potassium depolarization was complete. Figures 1 to

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The results described here were first presented by the author at the Fall Meetings, American Physiological Society, 1960 (Winegrad, S.: Physiologist 3: 179, 1960) and are to be presented in detail (Winegrad, S., and Shanes, A. M.: Calcium transfer and contractility in guinea pig atria. In preparation.).

3\* are taken from the work of Niedergerke,<sup>7</sup> and Niedergerke and Lüttgau.<sup>6</sup> Figure 1a and b shows the effect of replacement of sodium in the bathing solution at the beginning of a potassium-induced contracture. In figure 2a† the ventricle was depolarized by 100 mM potassium in the presence of 10 mM calcium, while in figure 2b the initial depolarization occurred in 0 Ca, 100 mM potassium but after 90 seconds the solution was changed to one containing 10 mM Ca, 100 mM potassium. The effect of the calcium is clearly not limited to processes accompanying depolarization. Similarly the effect of decreased sodium concentration can occur after depolarization (fig. 3). Electrical measurements eliminated increased depolarization as an explanation for the increases in tension seen with elevated calcium or depressed sodium concentrations.<sup>8</sup>

By use of radioisotopes, Niedergerke and Harris<sup>9</sup> demonstrated that the changes in extracellular sodium and potassium concentration associated with increased twitch tension were accompanied by increased uptake of  $\text{Ca}^{45}$  by the resting tissue (fig. 4‡) and by the tissue in potassium-contracture<sup>10</sup> (fig. 5§).

\*Figures 1 and 3 are reproduced from Niedergerke and Lüttgau: *Nature* 179: 1066, 1957.<sup>6</sup> By permission of the author and *Nature*.

†Figure 2 reproduced from Niedergerke: *J. Physiol.* 134: 584, 1956.<sup>7</sup> By permission of the author and the *Journal of Physiology*.

‡Figure 4 reproduced from Niedergerke and Harris: *Nature* 179: 1068, 1958.<sup>9</sup> By permission of the authors and *Nature*.

§Figure 5 reproduced from Niedergerke: *Experientia* 15: 128, 1959.<sup>10</sup> By permission of the author and *Experientia*.

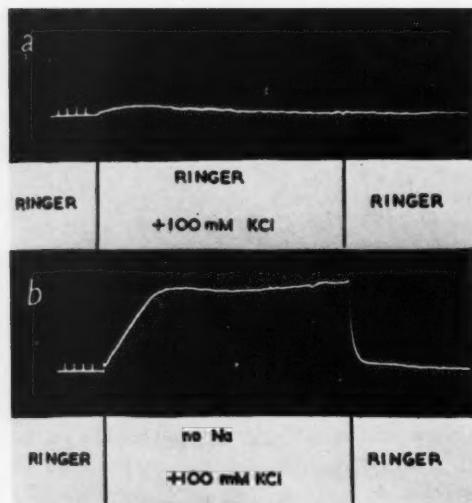


Figure 1

Dependence of contraction on sodium chloride concentration. Contractions always induced after a 30-min. period of equilibration in Ringer's fluid during which strip was stimulated at a constant rate. Ringer's fluid and potassium-rich solutions contained 0.5 mM calcium chloride throughout the experiment. Diameter of strip: 0.35 mm. (a) Contraction in the presence of 100 per cent sodium chloride induced by adding 100 mM potassium chloride to Ringer's fluid. (b) Contraction induced by adding 100 mM potassium chloride and simultaneously replacing the sodium chloride content of Ringer's fluid by sucrose. (From Niedergerke and Lüttgau.<sup>6</sup>)

These studies dealt primarily with rapid changes in calcium transfer; a distinction between changes occurring at the "cell surface" and in the "cell interior" based on a difference in time constants of calcium transfer was not clear.

The demonstration by Bianchi and Shanes<sup>11</sup> of an increased uptake of calcium during contraction of skeletal muscle fibers added substantially to the data implicating calcium as the excitation-contraction link. To test the possibility that excitation in myocardial cells is coupled with contraction by a movement of calcium into the cell during depolarization, a series of experiments comparing calcium uptake by guinea-pig atria at rest and under

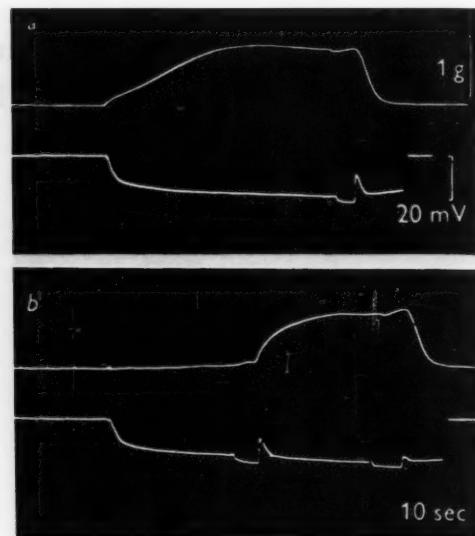


Figure 2

Effect of Ca on a depolarized strip, diam. 1.1 mm. In both records, upper traces, tensions; lower traces, depolarizations. The records started after soaking the strip in Ca-free Ringer's solution for 10 min. (a) Application of 10 mM Ca 100 mM KCl solution; (b) application, initially, of a Ca-free 100 mM KCl solution; 90 sec. later, 10 mM Ca was added to this solution, causing an immediate rise of tension. (From Niedergerke.<sup>7</sup>)

several different conditions of contraction was conducted. Radioisotope technics similar to those of Bianchi and Shanes<sup>11</sup> were used. Tensions were measured with conventional strain gauges. Left atrial appendages from young guinea pigs were used because the tissues are thin, stable over long periods of time, and devoid of spontaneous rhythm.

Calcium influx was determined by soaking the atria for 15 minutes in  $\text{Ca}^{45}$  solution and measuring the amount of  $\text{Ca}^{45}$  remaining in the tissue after the extracellular and loosely bound surface  $\text{Ca}^{45}$  had been washed out in nonisotopic solution. A correction was made for the intracellular  $\text{Ca}^{45}$  lost during the washout. To eliminate the error introduced by the presence of damaged tissue, the edges of the atria cut during dissection were removed from the undamaged tissue at the end of the experiment and their radio-

Table 1  
Relationship of  $Ca^{45}$  Uptake to Twitch Tension

$Ca^{45}$ conc. mmol/ml.	Rate beats/min.	Relative $Ca^{45}$ uptake, %*	Relative tension, %*	Number of exper.
1.25	15	37 $\pm$ 15	39 $\pm$ 9	5
2.50	6	30 $\pm$ 9	38 $\pm$ 5	6
2.50	15	92 $\pm$ 15	86 $\pm$ 9	14
2.50	30	100 $\pm$ 14	100	9
3.75	6	57 $\pm$ 29	58 $\pm$ 2	4
3.75	15	106 $\pm$ 14	105 $\pm$ 6	11

\*Values  $\pm$  1 standard error of mean.

activity counted separately. The effect of contraction on influx was measured by stimulating the muscles during the last 10 of the 15 minutes in  $Ca^{45}$ -Krebs solution and calculating the difference in  $Ca^{45}$  content between the stimulated and unstimulated muscles.

The resting influx at external calcium concentrations of 1.25 mM, 2.50 mM, and 3.75 mM was  $0.008 \pm 0.0010$ ,  $0.014 \pm 0.0012$ , and  $0.022 \pm 0.0046$  micromicromole/cm.<sup>2</sup>sec. In figure 6 the influxes are plotted against the external calcium concentration. The data can be best approximated by a straight line that, when extrapolated, misses the origin by a statistically insignificant amount. It would appear, therefore, that in the resting muscle cell, influx of calcium is a function of the external calcium concentration. The resting outflux, in 2.5 mM calcium, calculated by standard desaturation studies, was approximately equal to the influx, suggesting that in the resting atria no net movement of calcium was occurring.

When the atria contracted, the calcium influx increased considerably; the total calcium<sup>45</sup> content of the stimulated tissue at the end of the experiment rose to as much as 15 times that of the resting controls. Under these circumstances the increment in calcium influx associated with each beat was 0.57 micromicromole/cm.<sup>2</sup> surface area. Contraction was not accompanied by an increase in calcium uptake by the cut edges of the tissue, which themselves did not contract.

It was of interest to determine whether

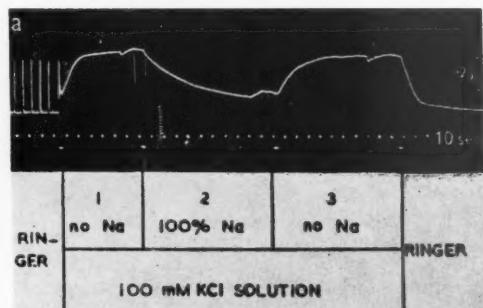


Figure 3

Reversibility and specificity of the action of sodium ions. Ringer's fluid and potassium-rich solutions contained 2 mM calcium chloride throughout the experiment. Diameter of strip, 0.45 mm. (1) Contracture induced by adding 100 mM potassium chloride to Ringer's fluid and simultaneously replacing sodium chloride iso-osmotically with sucrose; (2) reapplication of sodium chloride; (3) replacement of sodium chloride by sucrose. (From Niedergerke and Lüttgau.<sup>8</sup>)

any correlation existed between the size of the calcium influx per beat and the twitch tension. Table 1 consists of the results of experiments performed to study the effect of different external calcium concentrations and different frequencies of contraction on both the calcium uptake and the strength of contraction. Calcium uptake per beat and twitch tension for each condition are expressed in per cent relative to the values at a contraction frequency of 30/min. in 2.5 mM calcium, the latter having been arbitrarily assigned a value of 100 per cent.

The uptake per beat of atria contracting at 6/min. in 2.50 mM calcium is 30 per cent of that of the atria beating at 30/min. ( $p < 0.05$ ). The percentage increase in uptake per beat associated with this change in the rate of contraction does not differ significantly from the percentage increase in twitch tension. When the frequency is changed from 15/min. to 30/min., no significant increase in either twitch tension or uptake per beat occurs. Thus, in these experiments, both calcium influx and contractility apparently exhibit parallel saturation properties.

Muscles stimulated at 15/min. in 1.25 mM

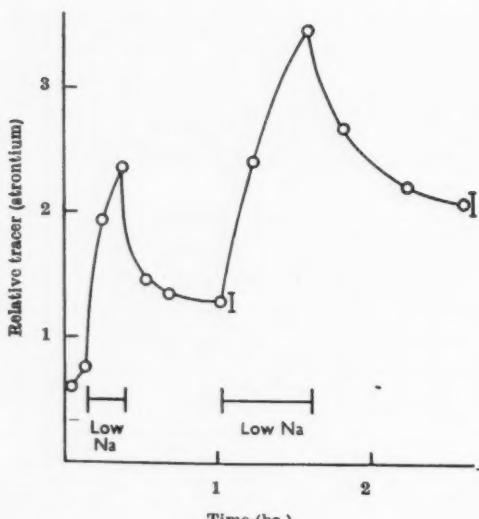


Figure 4

The effect of replacing the sodium chloride of Ringer's fluid by sucrose on the uptake of strontium-89 tracer in heart tissue. Relative amounts of strontium-89 tracer in a ventricle strip during exposure to tracer solutions which contained either 112.5 mM sodium chloride or 5 mM sodium chloride + 202 mM sucrose. The two solutions had identical concentrations of potassium chloride (2 mM) and of tracer Sr<sup>89</sup>-Ca (1 mM) mixture. Vertical bars indicate standard error of the measurement of radioactivity. Sr<sup>89</sup> is used as an indicator of Ca movement. (From Niedergerke and Harris.<sup>9</sup>)

calcium contract about as vigorously as the atria beating at 6/min. in 2.50 mM calcium. Despite the fact that two important conditions have been changed in opposite directions, the relative uptake per beat and relative tension are still closely correlated. An increase in external calcium concentration from 1.25 mM to 3.75 mM increases twitch tension almost 3-fold ( $p < 0.05$ ), and calcium uptake per beat is increased proportionately. An increase in the calcium concentration from 2.5 mM to 3.75 mM is associated with proportionately smaller increases in uptake and twitch tension, but in this comparison both changes are of questionable significance.

When a plot is made of relative twitch tension vs. relative uptake per beat using all the

data obtained from different external calcium concentrations and different rates of stimulation (fig. 7), a consistent correlation between the 2 parameters exists. A straight line closely fits the data and its failure to pass through the origin is not statistically significant.

These data demonstrating the increment in calcium influx associated with contraction are consistent with the hypothesis that calcium movement into the cell with depolarization couples excitation with contraction. They suggest, also, that such a link is a factor in determining the strength of the twitch.

In an analysis of the increment of calcium influx associated with contraction one may consider: (1) the source of this calcium; (2) the mechanism by which it enters the cell; (3) the time in the cardiac cycle during which the added calcium enters the cell; and (4) the possible mode of action inside the cell. Niedergerke's data<sup>9, 10, 12</sup> suggest that superficial sites in the resting cell bind calcium and that certain changes in the composition of bathing solution which produce increased twitch tension are associated with greater binding of calcium by the resting tissue. Moreover, experiments already mentioned show that similar changes in the composition of the bathing solution during a maintained potassium depolarization are rapidly followed by changes in contracture tension. An additional pertinent observation is that of Weidmann,<sup>13</sup> who demonstrated in turtle ventricle that a sudden increase in the concentration of calcium in the extracellular space during the initial stages of a twitch produced a more rapid rate of tension development and a greater peak tension than occurred at the lower calcium concentration. In addition, the increase in calcium concentration was accompanied by a shortening of the action potential. A hypothesis that incorporates these data with the observed correlation between the rate of calcium influx during a contraction and the size of the contraction would be the following: the calcium that enters the cell with contraction comes from

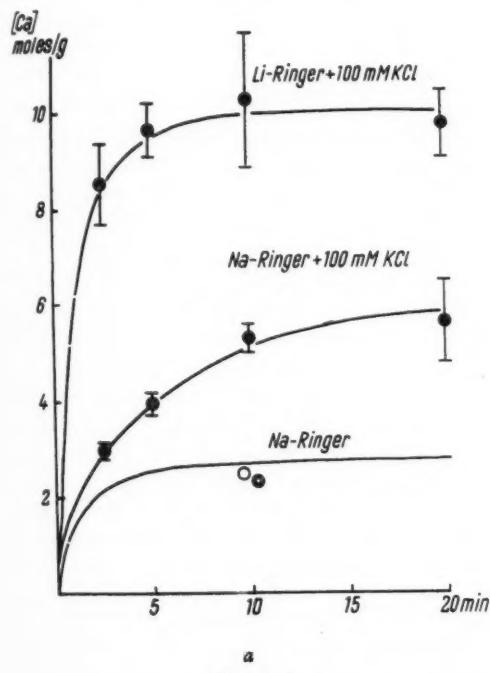


Figure 5

Effect of  $K$ -depolarization of  $Ca$  uptake of frog ventricular strips. The difference between the lower two curves shows additional  $Ca$  uptake due to 100 mM  $KCl$  Na-Ringer. Upper curve shows  $Ca$  uptake in absence of  $Na$  but with additional 100 mM  $KCl$ . Hollow circle and double circle represent two experiments showing  $Ca$  uptake in Ringer's solution made hypertonic by added 180 mM sucrose or added 100 mM  $LiCl$ . (From Niedergerke.<sup>10</sup>)

superficial sites and from the extracellular fluid; the calcium that enters the cell from the extracellular fluid during depolarization passes through the same superficial sites to which calcium was bound in the resting state.

Weidmann's data<sup>13</sup> suggest that the increment in calcium influx occurs during the depolarization. In support of this conclusion are the observations that the increased heart rate is associated with a shorter diastole, a shorter action potential but an increased calcium influx per beat, and an increased rate of rise of tension during the twitch.<sup>14-16</sup> If the influx of calcium is quantitatively related to twitch tension, it might be expected

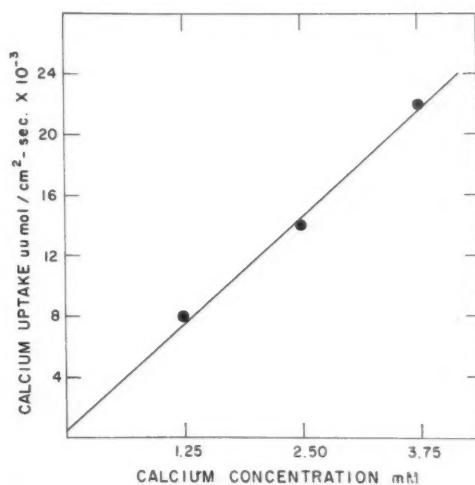


Figure 6

Calcium uptake of resting guinea-pig atrial appendage is plotted against extracellular calcium concentration.

that a greater increment during a shorter action potential would produce a more rapid rise in tension. If the added calcium does enter the cell during the action potential (measured to be 150 msec.), then calcium influx during depolarization may exceed resting influx by as much as 250-fold.

Certain possible relations of the calcium influx to contraction became apparent with a quantitative consideration of the data. If the molecular weight of myosin is about 500,000,<sup>17</sup> and its concentration in heart muscle is equal to that in skeletal muscle (taken to be 7.6 per cent of wet weight<sup>18</sup>) then each gram of heart contains  $1.5 \times 10^{-4}$  mmoles of this protein. The maximum increment in calcium influx measured in these experiments was  $0.6 \times 10^{-6}$  mmoles/Gm. The ratio of the number of calcium ions entering the cell during contraction to the number of myosin molecules already present is about 1/250. A similar ratio results if a comparison is made of the calcium influx to the amount of actin or tropomyosin in the muscle (again assuming that the concentration of actin and tropomyosin in heart muscle is similar to that in skeletal muscle). It is unlikely that only 0.4

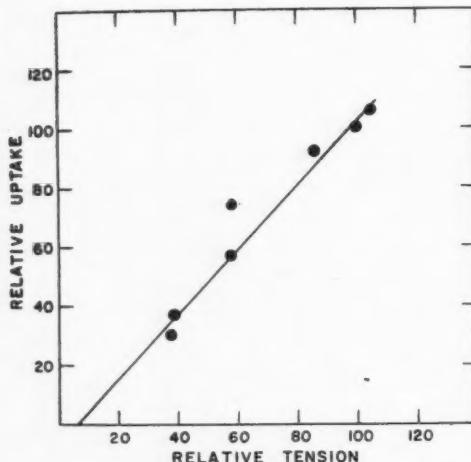


Figure 7

The relative calcium uptake per beat of guinea-pig left atrial appendages contracting at 6/min., 15/min., 30/min. in 1.25 mM, 2.50 mM, and 3.75 mM Ca-Krebs solution is plotted against the relative twitch tension produced. All values are expressed relative to calcium uptake and twitch tension at 30/min. in 2.5 mM Ca-Krebs solution.

per cent of the contractile protein is shortening during the strongest contractions of the isolated atrium. Therefore, if calcium does initiate the contraction, each ion must ultimately have an effect on many actin-myosin units. One of the reactions in the contractile process, though not necessarily the one involving calcium, must involve either a chain reaction or the interaction of 1 molecule or ion with as many as 250 molecules or units. The latter could occur by an enzymatic reaction or by a specific type of molecular alignment in which one molecule is in close association with many contractile units.

With respect to this quantitative relationship of calcium taken up during contraction to actomyosin, it is interesting to note the calculation of H. E. Huxley<sup>18</sup> that each thick filament in skeletal muscle contains about 400 myosin molecules. If a similar condition exists in the heart, the ratio of calcium ions taken up in a maximal contraction to the number of thick filaments is 1.6:1.

If the calcium that enters the cell with

contraction is assumed to achieve immediate uniform distribution in the cell water, the concentration would be  $1.2 \times 10^{-6}$  M. Any non-uniform distribution would produce regions within the cell of higher "calcium concentrations." This value of ionized calcium inhibits the relaxing-factor activity in vitro.<sup>19</sup> Ebashi has further shown that the relaxing-factor system binds calcium tightly and that procedures which decrease calcium binding proportionately decrease relaxation activity. He has demonstrated that other calcium chelating agents have relaxation activity proportionate to their ability to chelate calcium.<sup>20</sup> It is possible therefore that the calcium that enters the cell during excitation inhibits the relaxing system and thereby initiates contraction, or that it activates contraction, relaxation occurring by the removal of the calcium by the relaxing system.

#### Acknowledgment

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## Cardiac Active Principles in Blood Plasma

By STEPHAN HAJDU, M.D., AND EDWARD LEONARD, M.D.

A study of isolated frog hearts suggests that the amphibian heart is provided normally with a substance that maintains contractility and that disappears slowly from the heart during perfusion with saline solution. A search for substances that might fulfill this role in the frog heart has led to the isolation of many materials that have some degree of cardiotonic activity, but no conclusion can yet be reached about which, if any, of these substances is present in the intact frog heart. The search has led, however, to the discovery of 2 substances of mammalian origin that are as potent as the cardiac glycosides with respect to their inotropic action on frog heart. One of these is a phospholipid called lysolecithin, the other is a system of 3 plasma globulins called "cardioglobulin A, B, and C." The concentration of cardioglobulin C in man is increased in essential hypertension and aortic stenosis, 2 unrelated conditions that have in common the development of increased left ventricular isometric tension in systole. Conversely, cardioglobulin C is decreased in a group of patients with idiopathic congestive heart failure. The discovery of these substances is relevant to the question whether isolated mammalian cardiac tissue becomes hypodynamic in physiologic saline because of the loss of a system that helps maintain normal myocardial contractility. We have noted that most of the studies on isolated strips of mammalian cardiac tissue fail to answer this question, since the strips were probably hypodynamic because of impaired oxygenation or nonphysiologic saline media. Studies from our laboratory indicate that a slow decline in contractility on prolonged washing does occur in isolated mammalian heart tissue, despite good oxygenation and a normal environment with respect to inorganic ions. This can be prevented or reversed by perfusion with mammalian plasma. Along similar lines, it is of interest that, although the decline of performance characteristics of *in situ* mammalian hearts may be due to many factors, the decline can be prevented by perfusing the coronary system of the *in situ* heart with blood from a healthy donor animal. The problem, then, has 2 aspects. On the one hand, we must discover the physiologic significance of the potent glycoside-like substances already isolated from mammalian tissue; on the other, we must investigate the beneficial effects of plasma on heart strips or of donor dog blood on *in situ* hearts. Do these actions occur because of an effect on myocardial metabolism or do they come about because of a plasma substance that enhances myocardial contractility directly?

**A** WIDE VARIETY of experience has led to the conclusion that blood plasma has a beneficial effect on cardiac contractility. Thus, cardiac function declines when hearts are bathed in artificial saline media and improves when fresh whole blood or plasma is added. This effect was first noted by Ringer in 1885.<sup>1</sup> Recent interest in this phenomenon stems from two different lines of interest. On the one hand, now that much information has been obtained about the contractile protein of muscle and about the metabolic events that yield energy for contraction, it is appropriate to consider the problem of how the force of muscle contraction is regulated. The same question about the regulation of muscular

contraction presses to the fore in the field of cardiovascular research. We may mention as examples the abnormal increase in arteriolar tone in essential hypertension, the congestive heart failure that occurs in a number of patients in the absence of any known structural or inflammatory disease of the myocardium, and the cardiovascular collapse that often supervenes in patients subjected to a prolonged period of extracorporeal circulation. All these instances are characterized by abnormalities of involuntary muscle function, despite the absence of any gross anatomic or metabolic defects that might be invoked as causes of the disturbance. In this lecture we will review some experimental situations in which alterations in cardiac function might be due to the addition or depletion of cardiac active substances of biologic origin.

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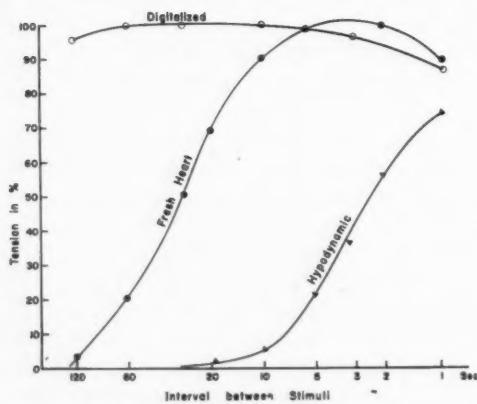
We shall begin with the isolated amphibian heart. The decline of myocardial contractility that occurs when hearts are bathed in artificial media was studied in detail by A. J. Clark in 1913;<sup>2</sup> he said that "excised frog hearts after perfusion for a few hours pass into a hypodynamic state in which both the force of contraction and the rate of conduction are markedly impaired." He concluded that the development of a hypodynamic state was associated with the loss of some essential substance from the frog heart, which was washed away slowly by prolonged contact with large volumes of saline solution.

There are several features about the hypodynamic heart that we would now like to enumerate. First, we may note that, immediately following excision and immersion in saline, a slow decline in contractility begins that continues steadily for a number of hours. The rate of decline depends to some extent on the thoroughness of the washing, that is to say, on the volume of perfusing solution and frequency of exchange. The first signs of the decline cannot be detected at high rates of stimulation but can be observed in the form of decreased twitch tension at lower frequencies. It should be emphasized that this decline occurs despite an environment that is ideal with respect to inorganic ions and oxygenation. Unlike mammalian heart muscle, the frog heart has no coronary system and depends for oxygenation on the movement of blood through the sponge-like network of muscle cells that constitute the ventricular wall. It is therefore no problem for oxygenated Ringer solution to perfuse the various parts of the frog ventricle adequately. Not only is there no impairment in oxygenation, but it is likely that the development of the hypodynamic state occurs in the absence of any decrease of high energy phosphate. Although this has not been studied in frog ventricle, Furchtgott and de Gubareff<sup>3</sup> have shown that the development of the hypodynamic state in guinea-pig atria is not associated with any change in the concentration of high energy phosphate compounds in the atrial muscle. It would appear therefore that

the hypodynamic state in frog heart muscle is not due to a defect in the supply of phosphate energy to the contractile protein. For a more detailed discussion of the question of whether the defect in experimental hypodynamic states is due to a defect in energy supply or energy utilization see reviews by Hajdu and Leonard<sup>4</sup> and by Wollenberger.<sup>5</sup>

We may now consider the substances that can restore frog-heart contractility to the original level. Actually, a very great number and variety of substances of plasma origin can improve the hypodynamic heart. Ringer concluded that the plasma activity resided in a nondialyzable fraction. Clark found that a positive inotropic effect could be obtained not only with soaps, such as sodium oleate, but also with various phospholipids and even amino acids. Other surface-active substances, such as bile acids, also improved contractility. In addition, beneficial effects have been obtained with adrenalin,<sup>6</sup> adenosine triphosphate (ATP),<sup>7</sup> and with pharmacologic concentrations of certain steroids, such as deoxycorticosterone and progesterone.<sup>7</sup> A great many investigators have worked in this field, and the interested reader can find more references in the review by Amberson.<sup>8</sup>

Most of the substances we have mentioned are capable of bringing the contractility of the hypodynamic frog heart back to normal, if they are added to the bathing solution in sufficient concentration. Their effect can be appreciated by reference to figure 1, in which twitch tension is plotted on the ordinate and the time interval between stimuli is plotted on the abscissa. The twitch tension of both the fresh heart and the washed hypodynamic heart varies with the frequency of stimulation. Comparison of these curves shows that, whereas the twitch tension of the hypodynamic heart approaches that of the fresh heart at high frequencies, at intermediate frequencies fresh heart is capable of developing much greater twitch tension than is the hypodynamic heart. The beneficial substances we have discussed above cause the hypodynamic curve to shift back toward the fresh heart curve.



Effect of prolonged saline perfusion on isolated frog hearts.

In one respect, however, the hypodynamic frog heart, which is improved by the addition of these materials, is still different from a normal heart: on subsequent washing, the hypodynamic state develops very rapidly. This is in marked contrast to the slow and gradual decline in contractility that occurs with a fresh heart. It seems reasonable to postulate that the development of the hypodynamic state in the frog heart is due to the slow loss of some material from the muscle. One would suspect that it is present in low concentrations in frog blood, and that an appropriate cardiac content is maintained because of the constant perfusion and the high affinity of the material for the heart. If so, perfusion of a hypodynamic frog heart with a large volume of normal frog plasma should result in the slow accumulation of the material, so that once again the heart would become hypodynamic only after prolonged washing.

Recently, a material that may fulfill these conditions has been isolated.<sup>9</sup> It is a phospholipid called " $\beta$ -palmitoyl-lysolecithin," which can be found in small concentrations in mammalian plasma. It has been isolated from adrenal medulla and may be rather widely distributed in the chromaffin system. Although this substance is present in mammalian plasma in concentrations too low to affect the hypo-

dynamic frog heart immediately, it becomes bound to frog cardiac muscle so that, if a frog heart is perfused with successive changes of serum, a significant amount of lysolecithin gradually accumulates. And, in contrast to the other substances discussed, after exposure to this material the restored heart becomes hypodynamic only gradually in the course of prolonged washing. Attempts have not yet been made to isolate lysolecithin from frog tissues. If it is found in frog plasma, it would be reasonable to postulate that lysolecithin is the naturally occurring substance that is slowly washed away from the frog heart during the development of the hypodynamic state.

Lysolecithin differs in another way from substances previously shown to restore the contractility of the hypodynamic frog heart. This can be appreciated by referring once again to figure 1, which shows how twitch tension varies with the interval between stimuli. Lysolecithin, like the cardiac glycosides, can cause maximal twitch tension even at very low rates of stimulation, which is represented in the figure by the nearly horizontal line at the top of the graph. In higher or toxic concentrations, the glycosides or lysolecithin induce systolic arrest or contracture, a phenomenon that is not seen with the other substances we have been discussing. Their positive inotropic effect is therefore profoundly greater than that of any of the other substances. Recently we have isolated from mammalian plasma a cardiotonic protein system of great potency that is comprised of three globulins that have been called cardioglobulin A, B, and C.<sup>10</sup> The effect of cardioglobulin on the frog heart in a concentration comparable to that found in normal human plasma is similar to that of a nontoxic concentration of cardiac glycosides. (This protein system also causes constriction of the peripheral vasculature of the frog in a Trendelenburg preparation, and therefore the vasoconstrictor globulin studied by Sakai and Hiramatsu many years ago may have been cardioglobulin.<sup>11</sup>)

A new question of great interest now arises as a result of these studies of the hypodynamic

amphibian heart. The discovery of 2 inotropic systems, lyssolecithin and cardiolipin, with cardiotonic potency comparable to that of the cardiac glycosides, indicates that we must determine whether either of these systems is important in the maintenance of normal myocardial contractility in the mammal. In this regard, the plasma concentration of cardiolipin in various clinical states is of interest. We have compared the plasma concentration of cardiolipin C in normals, in patients with aortic stenosis, and in patients with essential hypertension. In both aortic stenosis and essential hypertension, 2 conditions characterized by increased left ventricular isometric tension in systole, the concentration of cardiolipin C is significantly increased above normal.<sup>12, 13</sup> This is consistent with the idea that cardiolipin could be a naturally occurring cardiotonic system that is increased in the hyperdynamic states noted. The increased cardiolipin seems to be related to the increased pressure developed by the left ventricle and not simply to increased work, since in aortic insufficiency (characterized by increased stroke work without an increase in pressure), cardiolipin is normal. The concentration of cardiolipin has also been measured in various types of cardiac failure, and it has been found that values for patients with failure secondary to valvular disease (aortic or mitral insufficiency) are normal. In contrast, about half of the patients with cardiac failure secondary to idiopathic myocardial disease appeared to fall into a separate population with extremely low values of cardiolipin.<sup>12, 13</sup> The question whether the myocardial failure of this group may be caused by the observed cardiolipin deficiency cannot be answered at this time.

The questions about cardiolipin raised by these clinical correlations lead us to consider studies on experimentally induced hypodynamic states in mammalian heart muscle. Investigations on surviving strips of mammalian heart received considerable impetus when Cattell and Gold introduced the cat papillary-muscle preparation for the study

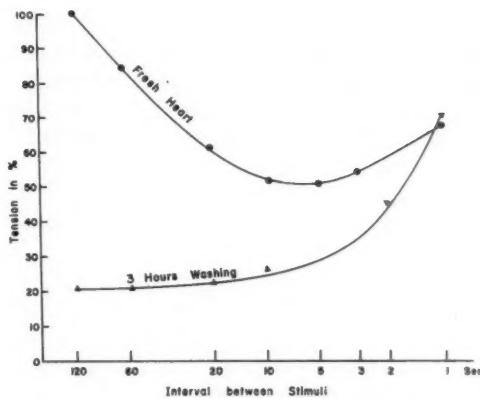


Figure 2

Effect of prolonged saline perfusion on rat right ventricle strips.

of the action of cardiac glycosides.<sup>14</sup> Since that time a great variety of substances of mammalian origin have been found to increase the contractility of isolated cat papillary muscle: including phospholipids,<sup>15</sup> amines,<sup>15</sup> amino acids,<sup>15</sup> serum albumin,<sup>16, 37</sup> a dialysate of serum albumin,<sup>17</sup> and various adrenal steroids.<sup>18</sup> The substances studied have been isolated not only from serum<sup>16, 17</sup> but also from fractions of dried spleen<sup>18</sup> and from liver.<sup>15</sup>

In attempting to evaluate these various results, it is pertinent to recall that the contractility of the isolated frog heart in saline declines slowly and steadily over a period of hours, despite normal energy metabolism and ionic environment, suggesting that a substance which maintains normal contractility is gradually washed away from the heart. Does a comparable situation exist in the case of mammalian hearts? Unfortunately the studies under consideration cast no light on this question because the comparable experimental conditions do not exist in the mammalian heart preparations. The mammalian tissues studied were hypodynamic because of abnormally low calcium or bicarbonate concentration in the extracellular medium in some cases, and in others it is probable that impaired oxygenation caused the hypodynamic state. In contrast to the frog heart,

which has no coronary system, oxygenation of dense mammalian ventricular tissue is difficult to accomplish once the coronary circulation is interrupted. Because of the difficulty in oxygenation, we would not expect cat papillary muscle stimulated at rapid rates to survive without some metabolic abnormality. Tanz, for example, has recently published photomicrographs showing severe histologic damage in cat papillary muscle stimulated at 1 per second at 37°C. for 6 hours.<sup>19</sup> Therefore, although a great variety of substances of biologic origin have been found to improve the performance of mammalian heart preparations, it is difficult to judge the physiologic significance of these findings, and none of the studies provides an answer to our question about whether there is a cardiotonic material bound to mammalian cardiac muscle that is lost when the tissue is removed from its normal environment.

Some recent results of our own, which are addressed to this problem, can be seen in figure 2. The studies were made on thin strips of right ventricle from very young rats, and the graph shows twitch tension plotted as a function of the interval between stimuli. The upper curve represents the normal pattern found in freshly prepared rat ventricle. After 3 hours of washing in Krebs bicarbonate solution, the lower curve is obtained, with considerably lower twitch tension over a wide frequency range. This can be reversed with digitalis or mammalian plasma, and the original decline can be prevented if the strip is maintained from the beginning in mammalian plasma. In general, it appears that mammalian cardiac muscle strips immersed in an environment which is ideal with respect to inorganic ions and oxygenation becomes hypodynamic on prolonged perfusion, and it is probable that this decline in contractility can be prevented if heart strips are maintained in mammalian plasma.

We will now turn to a brief consideration of studies on mammalian hearts *in situ* in which the vascular connections between the heart and other organs are partly or com-

pletely severed so that it is possible both to achieve some control over factors that affect myocardial function and to measure the rate of utilization of oxygen and other metabolic substrates. The prototype preparation is, of course, the heart-lung preparation, but there are also various interesting modifications in which 1 or more organs of the body are excluded from the general circulation. It was clearly stated by Starling and Visscher<sup>20</sup> that the classical heart-lung preparation deteriorates over a period of several hours, a decline which is reflected in both a decrease in contractility and in efficiency. It is apparent that many factors may contribute to the decline of myocardial performance in the heart-lung preparation. For example, a period of impaired coronary blood flow is almost inevitable and, if prolonged, will produce irreversible myocardial damage. The heart-lung preparation is deprived of sympathetic tone and probably undergoes progressive depletion of the cardiac sympathomimetic amines, which are an important determinant of myocardial contractility. Various substances produced by other organs of the body will not be available to the heart-lung preparation and these may conceivably be of importance at the level of either energy production or of energy utilization. Finally, disturbances may be produced by the tubing of the extracorporeal part of the system, and emboli may arise either from small clots or from bits of dried blood that form on the walls of the venous reservoir. In fact, one cannot overemphasize what a poor performance the heart-lung preparation renders compared to the intact heart. (For interesting recent data see references 21, 22, and 23.)

Although it is therefore practically impossible to find one's way among the various difficulties inherent in these preparations, 1 or 2 facts of interest stand out. In the first place, it becomes apparent from the results of several groups of investigators that myocardial performance is better when the liver and spleen are included in the circulation.<sup>22, 24-26</sup> In some studies the effect of liver and spleen appear to be primarily on the

metabolism of the heart,<sup>22, 24</sup> whereas in others some factor from the liver and spleen seems to have a primary effect on myocardial contractility.<sup>25, 26</sup> The nature of such a substance isolated from hepatic venous blood after stimulation of the splenic nerve has been said by Schmier to be a polypeptide.<sup>26</sup> Recently Sayers' group has suggested that hormones from the adrenal cortex may delay the decline of performance seen in a heart-lung preparation of the rat.<sup>27</sup> These findings, like those reviewed for isolated cardiac muscle, must be evaluated by asking why the heart-lung preparation is depressed in the first place, and whether the beneficial substances reported are important only in these experimental protocols or whether they have a general physiologic significance.

A new and encouraging note in the field of *in situ* heart preparations has been introduced by perfusing the coronary system of the experimental heart with blood from a healthy donor dog.<sup>23, 28</sup> This preparation, which has been studied by Sarnoff and co-workers, sometimes maintains a steady level of contractility for several hours. Therefore, despite all the difficulties that we have enumerated, it is possible for a denervated heart to maintain a fair level of functional capacity over a period of time, provided that vascular contact with a normal donor dog is maintained. One cannot say whether one of the beneficial effects of the donor animal is the maintenance at normal levels of some hypothetic cardiac active substance, but at least this preparation might provide a basis for experimental investigation of the point.

It would be of great interest, for example, to determine cardioglobulin concentrations in such a preparation. We know in the case of the rat that an extracorporeal circuit that includes a filter with a large air-blood interface is associated with rapid destruction of cardioglobulin C. A comparable destruction occurs in patients subjected to a period on a heart-lung machine. Whereas there is a comparatively small change in cardioglobulin C concentration during the pumping period of 30 to 45 minutes, we have found that

there is a marked drop in cardioglobulin concentration during the first few hours after surgery. During the ensuing 24 hours considerable recovery occurs, with cardioglobulin concentrations again approaching normal.

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## Discussion

**Dr. DeHaan:** Dr. Hoffman, I should like to ask a question—or 2 questions, as a matter of fact. First, do you believe that this very interesting disturbance, apparently of the all-or-none response of the bundle or bundle branches negates Curtis and Travis's findings in 1951 of the all-or-none response?

**Dr. Hoffman:** No, I don't think our observations negate the findings of Curtis and Travis (*Am. J. Physiol.* 165: 173, 1951). If a portion of the bundle or false tendon is stimulated and the action potential propagates in tissue that is fully repolarized, conduction will be all-or-none. However, if there are local differences in action-potential duration and if, for this reason, propagation enters partially depolarized fibers, decremental conduction of block may occur.

**Dr. DeHaan:** May I go on to the second question? In your closing comments, you mentioned a graded transition from conduction cells to typical ventricular cells. I assume that you were referring to the electrophysiologic transition; that is, the gradual change from 1 type of action potential to another. Or did you have some other evidence that the transition is really a graded one and not individual differences between cells in a fiber.

**Dr. Hoffman:** I was thinking of both. If you penetrate many fibers in the region of the junction, you get action potentials that are typical of Purkinje fibers, typical of ventricular muscle, and also of every conceivable intermediate form. Also, I thought that Kugler and Parkins (*Anat. Rec.* 126: 335, 1956), among others, had studied the histology of this region and shown at least 3 branches in the peripheral Purkinje fibers, all in nice focus in the same section. I think they called these branches A, B, and C; they look as if a gradual progression were present. Except for a few slides of our own, their anatomic studies are what I had in mind.

**Dr. Rhodin:** It is very satisfying to learn that our anatomic findings and Dr. Weidmann's physiologic ones concerning the number of cell divisions and their role in the

spread of excitation agree so well. There is one bit of information I should like to add to what I said yesterday. In comparing the intercalated discs in the myocardium and what I call the desmosome type that we find in the cells of the specific tissue, as perceived in the bundle of His and its branches, the surface of contact is increased at least by a factor of 50, so this, again, I think, would explain that the impulse travels so much faster in the specific tissue.

**Dr. Weidmann:** In which kind of cells is the increase—Purkinje or myocardial?

**Dr. Rhodin:** Purkinje fibers.

**Dr. Paul F. Cranefield** (Brooklyn, N. Y.): Dr. Weidmann's work on the diffusion of potassium is very elegant and important. The membrane at the intercalated disc is obviously there anatomically, and, ever since this has been known, there has been a tendency for people to assume that the disc is a barrier to conduction. Its anatomic presence does not, of course, prove that it has any electrophysiologic function.

I feel that, if the disc were to act as a direct impediment to conduction, then it should have a high resistance to the diffusion of ions; conversely, if it did have a low resistance, then it is hard to imagine how it could slow down propagation in any way. There is, in my opinion, as of now, no acceptable direct evidence that there is any such barrier to ionic movement.

I have noticed in the last few years a sort of willingness, almost a desire, to assume that this disc is a barrier to conduction. If we are forced to accept the disc as an impediment to conduction, then we will be faced with reconstructing all the electrophysiology of the heart on a radically new basis. I would urge all those who are not actively engaged in research bearing on this controversy to use caution and discretion in adopting this hypothesis as a possible explanation for their own observations.

**Chairman Brooks:** Of course, there is another view also creeping in, I think; that

is, that the disc aids conduction. I agree that this question of disc effect must be handled with discretion.

The first item in the next discussion is a 4-minute motion picture film, which will be presented by Dr. Podolsky.

**Dr. Podolsky:** Dr. Winegrad and Dr. Bianchi showed that calcium is made available to the muscle cell when it is stimulated. The question then is, what happens next? One way of finding out is to puncture the membrane with a micropipette and inject different solutions in the cell. This technic was first used by Heilbrunn and Wiercynski (*J. Cell. & Comp. Physiol.* 29: 15, 1947) and later by Niedergerke (*J. Physiol.* 128: 12 P, 1955), and they got striking results by injecting calcium.

Another preparation, which is considerably more flexible, was invented by the Japanese physiologist, Natori (*Jikeikai M. J.* 1: 119, 1954). He showed that the membrane of a single muscle fiber could be removed by microdissection and that the preparation was responsive to applied solution.

I should like to show some experiments made with Natori's preparation which demonstrate that it responds to calcium but not to magnesium, sodium, or potassium.

(Showing of the film, with the following comments by Dr. Podolsky.)

The membrane has been removed from the fiber and the myofibrils are immersed in mineral oil. An ordinary light microscope is used for observation. The striations are quite obvious. The pipettes are loaded with various solutions. Drops can be formed at the tip of the pipette and then applied to the myofibril. Since the pipettes are loaded with different solutions, the responses can be compared.

The first sequence shows a region where the membrane has been taken off and another where the membrane is intact. The test solution contains calcium at 3 mM. When drops are applied to the region *with* membrane, there is no response. Then, if they are applied to the *stripped* region, there is a contraction that is completely reversible.

The next experiment shows that, if calcium

is omitted, there is no response. Contraction is elicited only in that region of the myofibril which is in contact with the calcium solution. There seems to be no spread of the contraction.

The last sequence shows that, if calcium is replaced by magnesium, there is no contractile response. Also, sodium and potassium, at concentrations of 140 mM., do not trigger off the contractile mechanism.

**Chairman Brooks:** At Dr. Weidmann's request, I should like to recognize some people from Cleveland who are working on this problem.

**Dr. T. Hoshiko** (Cleveland, Ohio): I should like to draw attention to the work of Dr. Rothschuh (*Arch. ges. Physiol.* 225: 238, 1951) and to some work done with Drs. Sperekakis and Berne (*Am. J. Physiol.* 198: 135, 1960; *Am. J. Physiol.* 198: 531, 1960; *Fed. Proc.* 19: 108, 1960; *Proc. Soc. Exper. Biol. & Med.* 101: 602, 1959) that, we feel, show up some difficulties in the "syneccytial" theory: (1) Dr. Rothschuh showed that, in cardiac muscle, depolarization upon injury will spread for only a short distance, less than 1 mm. In skeletal muscle, such depolarization will gradually spread over the whole length of the muscle. (2) In frog heart perfused with hypertonic solution, quiescent cells with normal resting potentials were found adjacent to very active cells. (3) The resistance of the single frog ventricular cell was high, namely, 12 megohms. The resistance between 2 cell interiors was approximately double this value. (4) We have induced unidirectional propagation in a predictable direction in frog ventricular strips; this phenomenon cannot be easily explained in terms of a functional syncytium. (5) We found that tissue DC resistance of heart muscle increased 7-fold when the interspace ion concentration was reduced by one-tenth; on the other hand, in sartorius muscle, where the cells extend almost the whole length of the muscle, the resistance increase was only 2- to 3-fold.

Finally as shown in figure 1, we measured the impedance of cat papillary muscle strips at various frequencies, before (open stars)

and after (solid stars) interspace ion depletion. The initial impedance at 10 cps was set equal to 1. These were compared with the impedance of sartorius muscle before (open circles) and after (solid circles) soaking in isotonic sucrose for about 1 hour. Before soaking in sucrose, sartorius muscle impedance was independent of frequency, while cardiac muscle impedance fell slightly at the higher frequencies. After soaking in sucrose, sartorius muscle impedance rose 2-fold and was still independent of these frequencies. On the other hand, cardiac muscle impedance rose 10-fold at the low frequency and fell drastically at the higher frequencies. At the higher frequencies, a high resistance appears to be short-circuited by a capacitive component.

These facts, taken together, appear to indicate the presence in cardiac muscle of transverse membranes of respectable resistance and capacitance, which probably are the intercalated discs. High resistance discs would make the functional syncytium theoretically inadmissible.

**Dr. Weidmann:** I will be very brief. I think that these membranes have some resistance and I am quite sure that they represent a capacity. The point of the argument was really a quantitative one rather than a qualitative one. I think we have to go privately through all of the data and see what can be done.

**Dr. Eichna:** I guess I don't understand the structure of the intercalated discs. Is the intercalated disc part of the same membrane that forms the wall of the cell or is it not?

**Dr. Weidmann:** It is part of the same membrane. The membrane wrapping the side of the cell toward the extracellular space continues directly into the disc, so I guess it is the same material. But the functional significance of 2 apposed membranes may not be the same as that of 1 membrane in contact with the extracellular space. After all, the ionic composition might be different, and this difference may change the resistance of the membrane.

**Dr. Bing:** I should like to comment on Dr.

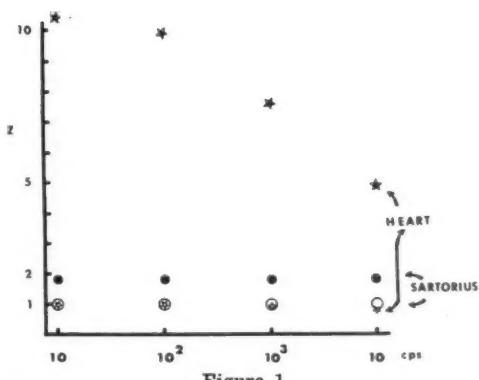


Figure 1

Relative impedance versus frequency: in Tyrode's solution,  $\circ$ ; in sucrose,  $\star$ . Ordinate=impedance; abscissa=frequency.

Hadju's observation that the hypodynamic state is at first observed only at low frequencies but that later, on further washing, it occurs even at high frequencies.

It is certainly true that the mechanical efficiency of the isolated heart is low. We were unable to restore normal efficiency by adding plasma from normal dogs to the perfusion fluid. We have ascribed the decline in mechanical efficiency of the isolated heart to depletion of catecholamines in the perfusion fluid. This increase in efficiency occurs upon addition of small quantities of catecholamines and upon the inclusion of liver and spleen in the perfusion circuit. However, the changes in efficiency did not result from increased cardiac work, but from a diminution in myocardial oxygen consumption. It seems to me that it would be nice to have Dr. Hajdu's cardioglobin defined more precisely.

**Dr. Hajdu:** To deal with your second point first, the addition of norepinephrine increases efficiency but does not restore cardiac contractility to normal. As to the failure of fresh blood to restore contractility, it is difficult by exchange-transfusion methods to increase the concentration of certain substances to normal. Our own experience with cardioglobulin bears on this point, since we have found that, in preparations which include an extracorporeal circuit, the concen-

tration declines rather rapidly and cannot be brought back by the exchange method.

*Dr. Olson:* I should like to ask Dr. Winegrad a question. With regard to your very interesting calculation of molar ratios of  $\text{Ca}^{++}$  to actomyosin, I take it you meant myosin and not actomyosin. Is that correct?

*Dr. Winegrad:* These calculations are very gross, and I will leave disagreement about the molecular weights to the biochemists. The figure of 500,000 was used as an approximation of the actomyosin unit.

*Dr. Olson:* But the measurements on actomyosin show that it is a much larger molecule. Although actin G is 70,000, actin F is about  $1.5 \times 10^6$  and the measurements on skeletal actomyosin show it to be an enormous molecule of the order of  $20 \times 10^6$  in weight.

*Dr. Winegrad:* That's right.

*Dr. Olson:* The thing that occurred to me is that calcium introduced in vitro might activate myosin ATPase, but in the intact fibril Perry (*Physiol. Rev.* 36: 1, 1956) has shown that magnesium is a more effective activator than calcium. The question of myosin-ATPase activation by ions is very interesting and still controversial. Even the movie revealing an inotropic effect of added  $\text{Ca}^{++}$ , which Dr. Podolsky showed, involves concentrations of calcium ions very much larger than the amount which you calculate move intracellularly during contraction. Isn't that true?

*Dr. Winegrad:* There are 2 considerations in this respect. First, since uniform distribution of the calcium entering the cell during contraction is probably not immediately achieved, regions with calcium concentrations higher than these calculations show will exist inside the cell transiently.

Second, with reference to the specific values of calcium concentration at uniform distribution, these values are of the same order that Dr. Ebashi (unpublished data) has found will affect the action of his relaxing factor system on *in vitro* actomyosin preparations.

Three experiments were performed in which the guinea-pig atria were stimulated at 60/minute. At this rate the atria could not maintain their contraction tension for 10 minutes without some decline. A decline in calcium uptake per beat also occurred in these experiments. These tissues have been irreversibly damaged and have suffered what one might call an acute type of failure, possibly related to the altered calcium metabolism.

*Dr. Olson:* I should like to say one thing about the origin of the heart failure of reduced energy utilization. All the evidence suggests that the oxidative process in this type of heart failure is normal. A multiple etiology may be involved: for example, there may be a disturbance in some aspect of chemical-mechanical coupling during contraction; or, other factors, such as Dr. Hajdu's plasma factor, or a variety of steroids, or the size and shape of the myosin molecule, may all be critical for the normal contractile cycle. It may very well be that ultimately we can classify the heart failure of reduced energy utilization into several subcategories.

*Chairman Brooks:* I was hoping we could end the symposium in a blaze of light rather than in an increasing darkness [referring to lights, which had just been turned on]. Now that the lights have come on again, our purpose is accomplished. In closing, I wish to thank the panel for their contribution to this meeting.

### The Pulsatile Beat of the Heart

I am obliged to conclude that in animals the blood is driven round in a circuit with an unceasing, circular sort of movement, that this is an activity or function of the heart which it carries out by virtue of its pulsations, and that in sum it constitutes the sole reason for the heart's pulsatile movement.—W. Harvey. *Movement of the Heart and Blood in Animals*. Translated by N. J. Franklin. Springfield, Ill., Charles C Thomas, 1959 p. 87.

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